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(54) Title: BREAD COMPRISING SOY PROTEIN

(57) Abstract: The present invention relates to bread containing exogenously added protein and optionally exogenously added dietary fibres and methods for preparation thereof. The exogenously added proteins are soy proteins and the exogenously added dietary fibres are soy fibres, more preferably soy cotyledon fibres.

BREAD COMPRISING SOY PROTEIN

FIELD OF THE INVENTION

The present invention concern bread containing exogenous soy protein and having an improved eating quality and also concern methods for manufacturing bread containing exogenously added protein and having an improved eating quality. Furthermore, the present invention relates to soy protein, phytoestrogens, phospholipids, and dietary fibers and breads incoporating same suitable for preventing, treating and/or alleviating cardiovascular diseases such as hypercholesterolemia, hypertriglyceridemia, hyperlipidemia, arteriosclerosis, hypertension and related cardiovascular diseases, for preventing and/or treating type 2 diabetes and/or the metabolic syndrome, and for preventing, treating and/or alleviating pulmonary diseases. The present invention also pertains to the use of such breads in the prevention and/or treatment of a cardiovascular disease in a subject suffering from type 2 diabetes

A bread according to the present invention is particularly useful in preventing and/or reducing the influx of triglycerides and/or cholesterol into the arterial wall and/or reducing the accumulation of cholesterol in the arterial wall of subjects at high risk for developing cardiovascular disease or subjects already suffering from a cardiovascular disease such as atherosclerosis or diabetic subjects. A bread according to the present invention is also useful for lowering serum levels of total cholesterol and/or LDL-cholesterol and/or triglycerides and/or homocystein and/or for increasing serum levels of HDL-cholesterol and/or for improving the serum HDL/LDL-ratio in subjects at risk for developing cardiovascular diseases and in subjects already suffering from an arteriosclerotic condition such as e.g. atherosclerosis or a related cardiovascular disease. A bread according to the present invention is also useful in lowering serum levels of total cholesterol and/or LDL-cholesterol and/or triglycerides and/or glucose and/or increasing serum levels of HLDL-cholesterol in diabetic subjects. A bread according to the present invention is also useful in treating e.g. chronic obstructive pulmonary disease (COPD), inflammation of the airways, asthma, bronchoconstriction, bronchitis, and small airways disease.

The present invention also concerns use of a bread according to the present invention in the prevention and/or treatment of said diseases and disorders and for lowering serum levels of total cholesterol and/or LDL-cholesterol and/or triglycerides and/or homocystein in subjects. In addition, the present invention also provides methods for

preventing, treating, prophylactically treating and/or alleviating by therapy said diseases and disorders.

BACKGROUND OF THE INVENTION

The field of functional foods has increased tremendously in recent years as the health awareness of the population in the industrialised parts of the world has gone up coincident with an increase in lifestyle related syndromes such as obesity, cardiovascular diseases and type II diabetes. Several studies show a significant correlation between the diet of a subject and the risk of the subject of contracting one or more of these diseases and syndromes. This has put the spotlight on the healthiness of everyday diet and as research into the field continues many items are added to the group of ingredients that should be present in a healthy diet. Functional foods can be defined as ordinary food items, which include one or more ingredients that can be beneficial to human health in one way or another. An example of a desirable functional food product may be a baked product, such as bread. This product does not require any cooking on the part of the consumer and can be eaten as it is.

Intake of soy protein, either alone or in combination with other soybean components, has been shown to have several beneficial effects on humans. Naturally, the soy protein could be consumed by eating soybeans, but this has little appeal, as soybeans are conceived by some to have an objectionable flavour and furthermore are not part of a traditional diet in the Western world. An alternative way of consuming soy protein is as part of a functional food product containing soy protein.

One way of consuming soy constituents is as a part of a bread product such as white, wholemeal or rye bread. Until now, there have been limitations on the amount of soy protein, phospholipids and fibres which can be incorporated into a bread product without affecting crust-structure, crumb-structure, bread volume and palatability. Previous attempts to incorporate levels of fibres and protein higher than approximately 5 weight percent into bread have failed. With conventional techniques, the addition of the amounts of soy protein, phospholipids and fibres required to obtain a significant nutritional benefit will make the resulting bread unpalatable.

GB901110 relates to baked goods and methods and compositions for producing the same, and is particularly directed to production of baked goods having an unusually high protein content. By introducing vegetable protein material to the ingredient

mixture in an undenatured form, with a major proportion of the protein having a molecular weight of >150,000 Da, preferably at least 40% having a molecular weight of 300,000 Da, and having reactively available sulfhydryl in an amount from 0 to $7x10^7$ moles per gram, the resulting bread has a protein content up to 11-20% and the visual and organoleptic characteristics of conventional, low-protein white bread. In preparing the dough, supplementary water equal to 50-100% of the weight of the protein supplement is employed.

US3930055 relates to ingredients and a process for producing baked products having a very low carbohydrate content yet with satisfactory density, texture, color, toastability, aroma and other desirable properties. More particularly the invention relates to baked products made from a dough consisting principally of proteinaceous powder, e.g. extracted from soybeans, said powder having less than 10% carbohydrate and leavening agent.

US5698256 relates to a baked product having a blood sugar reducing effect for diabetics, which is baked from a dough containing soy protein, a fiber component containing at least 50% by weight of wheat bran, vegetable oil, a fat-containing, substantially starch-free nut and/or kernel, a low-fat sour milk product, egg white and a leavening agent in specific ratios. The product according to the invention is described as bread-like.

GB388319 relates to a process for making bread for diabetics from soy beans, consisting in grinding non-disembittered soy beans, which have been de-oiled to the extent of about one half of the oil content, working up a gough without adding cereal flour, and baking the dough using 10 to 12% more fermenting agent than otherwise usual for making ordinary cereal bread.

US3650764 relates to enzymatic baking compositions and methods for using same.

JP4190735 relates to a process to produce voluminous, tasty bread with good water-holding property which can remain fresh for ca. 2 weeks comprising: combining deodorised soybean powder in flour; adding activator for yeast such as basic pentacalcium triphosphate, to the mixture, and preparing bread by conventionel process.

In "Getreide, Mehl und Brot, (1973) 27,5:154-160. [New experiences with the use of soy products in bread and bakery products]" describes the use of different soy products in bakery-products. 10-20% defatted toasted soy flour produced colour and flavour deterioration in mixed rye-wheat bread, but 10% soy semolina gave satisfactory results. A 5% addition of defatted heat-treated soy protein concentrate (<60% protein) reduced the quality of bread slightly.

US5192564 relates to a composite dough fro producing a baked product comprising an inner dough portion and an outer dough portion. The inner portion having less oven spring than the conventional leavened outer dough portion. The invention provides a means of adding to the inner dough a significant level of a fibrous additive such as soy polysaccharide material.

It has now been found that it will be possible to incorporate higher amounts, such as 12.5 weight percent, of soy protein (isoflavones/phytoestrogen) into a bread product by using a new process as described below.

SUMMARY OF THE INVENTION

The present invention provides bread with a high eating quality characterised by having a high content of exogenous protein and optionally of phospholipids and/or of fibres. The present invention also provides a method for manufacturing bread with a high eating quality characterised by having a high content of exogenous proteins and optionally of phospholipids and of fibres. Said method is further characterised by employing a blend of exogenous protein and optionally phospholipids and/or fibres, which may be hydrated prior to incorporation into the dough. According to said method additional liquid may also be added to the dough. It is believed that the hydration of the blend and the adjustment of the amount of liquid added to the dough counteracts the negative effect of hydrocolloids and proteinaceous material on bread quality. Such negative effects will be more pronounced if the bread further comprises high amounts of fibre. It is known that water has to be absorbed by the protein in the wheat flour in order to allow its transformation into gluten. If absorbent material is added to a formulation of a bread it takes away water from the protein, thereby reducing gluten development with the effect of impairing the quality of the baked bread.

Intake of a protein enriched bread according to the invention will provide any beneficial effects associated with the exogenously added proteins. Furthermore, a protein

enriched bread product according to the present invention will be able to comply better with consumer-expectations than bread products prepared by use of previously available methods for protein fortification of bread products, since bread with an increased eating quality is obtained by the hydration of the soy blend and the adjustment of the amount of liquid added to the dough. In addition, bread comprising soy tend to preserve moisture for longer periods than traditional bread, resulting in improved shelflife.

In one aspect, the present invention provides bread with a high eating quality characterised by having a high content of exogenous proteins and optionally of phospholipids and/or of fibres.

In another aspect, the present invention provides a method for manufacturing bread with a high eating quality characterised by having a high content of exogenous proteins and optionally of phospholipids and of fibres. Said method is further characterised by employing a blend of exogenous protein and optionally phospholipids and/or fibres, which may be hydrated prior to incorporation into the dough. According to said method additional liquid may also be added to the dough.

In another aspect, the present invention provides a product prepared by a method according to the present invention.

In yet another aspect, the present invention provides the use of a bread product with a high eating quality characterised by having a high content of soy proteins and optionally of phospholipids and/or of fibres for lowering serum levels of glucose and/or total cholesterol and/or LDL-cholesterol and/or triglycerides and/or homocystein and/or for increasing serum levels of HDL-cholesterol and/or the serum HDL/LDL-cholesterol ratio in a subject.

The exogenously added protein may be any type of protein or any combination of proteins and may consequently be provided by any protein source or by a combination of protein sources. Examples of exogenously added proteins in the context of the present invention are vegetable proteins, such as soy proteins, and animal proteins. The exogenously added protein is preferably soy protein. The soy protein is preferably provided by isolated soy protein, soy protein concentrate, soy flour or the like or any combination thereof. (The soy protein is preferably provided by isolated soy protein).

Isolated soy protein is the major proteinacious fraction of soybeans. It is prepared from high quality, dehulled, defatted soybeans by removing a preponderance of the non-protein components resulting in an isolated soy protein fraction which shall contain at least 80 (90) percent protein on a moisture free basis. Soy protein concentrates are made by removing most of the oil and water-soluble non-protein constituents from defatted and dehulled soybeans. In the present context a soy protein concentrate shall preferably contain at least 65 (70) percent protein on a moisture-free basis. The soy protein may also be provided by soy flour, which may be full-fat or defatted soy flour. Full-fat soy flour comes from whole, dehulled soybeans that have been ground into a fine powder and, as the name implies, still contains the fat naturally found in soybeans. Defatted soy flour comes from whole, dehulled, defatted soybeans that have been ground into a fine powder. Soy flour contains approximately 50 percent protein on a dry weight basis in the present context.

Preferred isolated soy protein products are supplied by Protein Technologies International, Inc. (under the brand name of *SUPRO®.)* A presently preferred quality is FXP HO 161, but the protein source may be another quality or a mixture of different qualities.

A protein source for use in a method according to the present invention comprises the source or sources of exogenously added proteins. The amount of protein in a protein source for use in a method according to the present invention and the amount of the protein source is preferably such that the amount of exogenously added protein in the resulting bread product is at least about 5 weight percent, such as at least about 6 weight percent, for example at least about 7 weight percent, such as at least about 8 weight percent, for example at least about 9 weight percent, such as at least about 10 weight percent, for example at least about 11 weight percent, such as at least about 12 weight percent, for example at least about 13 weight percent, such as at least about 14 weight percent, for example at least about 15 weight percent, such as at least about 16 weight percent, for example at least about 17 weight percent, such as at least about 18 weight percent, for example at least about 19 weight percent, such as at least about 20 weight percent, for example at least about 25 weight percent, such as at least about 30 weight percent, for example at least about 35 weight percent, such as at least about 40 weight percent, for example at least about 45 weight percent.

Optionally, bread according to the present invention may also comprise exogenously added dietary fibres. Examples of dietary fibres include fibres from apples, oats, and soybeans. The dietary fibres are preferably provided by soybean fibres, and more preferably by soy cotyledon fibres. Preferred soy cotyledon fibre products are supplied by Protein Technologies International, Inc. under the brand name of FIBRIM®. Among the various soybean fibres produced under the FIBRIM® brand, FIBRIM® 1020 is particularly preferred in the present invention. This product also contain soy protein in an amount of 12.4%. Other products of the FIBRIM® brand such as e.g. FIBRIM® 2000 or mixtures of fibre products may also be used.

Dietary fibres may optionally be incorporated in the resulting bread product in an amonut of at least 0.9 weight percent, such as at least about 1 weight percent, for example at least about 1.1 weight percent, such as at least about 1.2 weight percent, for example at least about 1.3 weight percent, such as at least about 1.4 weight percent, for example at least about 1.5 weight percent, such as at least about 1.6 weight percent, such as at least about 1.7 weight percent, for example at least about 1.8 weight percent, such as at least about 1.9 weight percent, for example at least about 2 weight percent, such as at least about 2.5 weight percent, for example at least about 3 weight percent, such as at least about 3.5 weight percent, for example at least about 4 weight percent, such as at least about 4.5 weight percent, for example at least about 5 weight percent, such as at least about 5.5 weight percent, for example at least about 6 weight percent, such as at least about 6.5 weight percent, for example at least about 7 weight percent, such as at least about 7.5 weight percent, for example at least about 8 weight percent, such as at least about 8.5 weight percent, for example at least about 9 weight percent, such as at least about 9.5 weight percent, for example at least about 10 weight percent, such as at least about 15 weight percent, for example at least about 20 weight percent, such as at least about 25 weight percent.

A protein source for use in a method according to the present invention may also optionally comprise carbohydrate sources, fat sources, flavouring agents, vitamins, minerals, electrolytes, trace elements and other conventional additives and the like. If a fat source is present in a protein source for use in *step i) of* a method according to the present invention, a preferred fat source is lecithin, especially soy lecithin. *When lecithin, or soy lecithin, is present in a protein source for use in step i) of a method according to the present invention, it is usually present in an amount of from about 0.5*

to 10 weight percent, preferably from about 2.5 to 6 weight percent of the total protein source.

Preferred examples of protein sources for use in a method according to the present invention are the compositions described in WO 97/31546, which are hereby incorporated by reference. Said patent application discloses compositions comprising (a) isolated soy protein, (b) soybean fibres, preferably soy cotyledon fibres, the amount of (a) being such that the protein content provides at *least 15%* of the total energy content of the composition, and the weight ratio between (a) and (b) being at least 2, *preferably at least 3.* These compositions are useful for lowering serum levels of cholesterol and triglycerides and for increasing the HDL/LDL-cholesterol ratio in subjects and for treating obesity.

Additionally preferred examples of protein sources are the compositions described in *PCT/IB99/01992, PCT/IB99/01997 and PCT/IB99/01998.* Said patent applications disclose compositions comprising (a) a soy protein source, selected from isolated soy protein, soy protein concentrate, or soy flour, of which isolated soy protein is most preferred, said soy protein source providing an amount of soy protein, which is at least 45 weight percent of the total protein content of the composition, preferably at least 50 weight percent of the total protein content of the composition, said total protein content providing at least *15 percent* of the total energy content of the composition, (b) at least one phytoestrogen compound in an amount of more than 0.10 weight percent of the soy protein content of the composition, and (c) dietary fibres, preferably soybean fibres, more preferably soy cotyledon fibres, in an amount of more than 4 weight percent of the total weight of the composition on a dry basis. These compositions are particularly useful for lowering serum levels of total cholesterol, LDL-cholesterol, triglycerides, homocystein, reducing the influx of cholesterol and/or triglycerides into the arterial wall, reducing the amount of oxidized LDL-cholesterol present in the arterial wall, increasing the serum HDL/LDL-cholesterol ratio and/or the serum level of HDL-cholesterol in a subject, including a diabetic subject, reducing and/or eliminating mucus hypersecretion and/or dyspnea in a subject suffering from asthma and/or increasing FEV1 of a subject as measured by forced expiratory volume in the first second of expiration. An example of these compositions is shown to be able to lower serum levels of *inter alia* LDL-cholesterol with as much as 13%. These compositions may therefore be effective in preventing, treating, prophylactically treating and/or alleviating diseases such as cardiovascular diseases, type II diabetes, cardiovascular

diseases in diabetics, the metabolic syndrome and pulmonary diseases as described in said applications.

One embodiment of the present invention provides methods by use of which the above-mentioned compositions may be incorporated into bread in amounts, which methods result in bread with increased amounts of exogenously added soy protein. Intake of a bread according to the present invention will provide the beneficial effects associated with said compositions. Furthermore, a bread prepared by a method according to the present invention will be able to comply better with consumer-expectations than bread prepared by use of previously available methods for protein fortification of bread.

One aspect of the present invention provides the use of *bread products* as described in the above paragraph for lowering serum levels of glucose and/or total cholesterol and/or LDL-cholesterol and/or triglycerides and/or homocystein and/or for increasing serum levels of HDL-cholesterol and/or the serum HDL/LDL-cholesterol ratio in a subject.

The present invention provides a bread comprising a protein source, preferably having a high, fixed amount of a phytoestrogen compound such as e.g. naturally occurring isoflavones, and a phospolipid source. More particularly the present invention provides a bread on basis of soybean extractable ingredients comprising soy lecithin, preferably having a high fixed level of phosphatidyl choline, and having a high, fixed amount of a phytoestrogen compound such as e.g. naturally occurring isoflavones.

The present invention provides a bread comprising a) soy protein, preferably isolated soy protein, b) a high content of a plant hormone in the form of a phytoestrogen compound, preferably naturally occurring isoflavones, (c) a phospholipid source, more preferably lecithin, and even more preferably soy lecithin and preferably having a high fixed level of phosphatidyl choline and optionally (d) dietary fibers, preferably soybean fibers, more preferably soybean fibers manufactured from the cotyledon of soybeans hereinafter referred to as soy cotyledon fibers and the present invention furthermore represents a potential new breakthrough in the treatment of cardiovascular diseases, diabetes and pulmonary diseases.

A bread according to the present invention is useful in treating including prophylactically treating cardiovascular diseases such as hypercholesterolemia, hypertriglyceridemia, hyperlipidemia and other cardiovascular diseases such as e.g. arteriosclerosis. It is one objective of the present invention to significantly lower levels of total serum cholesterol and LDL-cholesterol and triglycerides in a mildly hypercholesterolemic subject. It is another objective of the present invention to significantly lower serum levels of total cholesterol and/or LDL-cholesterol and/or triglycerides in a subject suffering from hypercholesterolemia and/or hyperlipidemia. It is another objective of the present invention to render the arterial wall more resistant to the accumulation of lipoproteins. It is a further objective of the present invention to provide a bread effective in preventing, treating, prophylactically treating and/or alleviating an arteriosclerotic condition by reducing the influx of cholesterol and/or triglycerides into the endocelium of the arterial wall and/or by causing the dilation of blood vessels. Yet another objective of the present invention is to reduce lipid plaque formation.

The present invention is also useful in the prevention and/or treatment of type 2 diabetes and/or a cardiovascular disease in diabetic subjects. Accordingly, it is an objective of the present invention to effectively lower serum levels of both glucose and cholesterol and/or triglycerides. No treatment is currently available for concomitantly lowering serum levels of glucose as well as lipid serum levels of total cholesterol and/or LDL-cholesterol and/or triglycerides. It is to be understood that diabetic subjects according to the present invention have a fasting plasma glucose $\geq$ 7.0 mmol/l.

A bread according to the present invention represents a new approach to treatment of type 2 diabetes and is believed to be capable of i) lowering total serum levels of cholesterol and/or LDL-cholesterol and/or triglycerides and/or increasing serum levels of HLDL-cholesterol, ii) increasing glucose tolerance and/or insulin sensitivity and/or, iii) lowering serum levels of glucose, iv) preventing, treating and/or alleviating impaired glucose tolerance and/or insulin secretory failure in diabetic subjects and/or v) preventing, treating and/or alleviating an arteriosclerotic condition by reducing the influx of cholesterol and/or triglycerides into the endocelium of the arterial wall of a diabetic subject suffering from a cardiovascular disease and/or by causing the dilation of blood vessels. No other known breads are effective in lowering serum levels of both lipids and glucose and/or reducing the influx of lipids such as e.g. cholesterol and/or triglycerides into the arterial wall.

The present invention is also useful in the prevention and/or effective treatment of pulmonary diseases such as e.g. airway inflammation, asthma, bronchitis and small airways diseases, in particular asthma including chronic asthma such as e.g. asthma characterized by a chronic inflammatory condition. The present invention is believed to be capable of increasing FEV_1 of a subject, measured by forced expiratory volume in the first second of expiration, as well as being capable of treating, alleviating and/or eliminating in particular i) inflammation of the airways, ii) mucus hypersecretion, and iii) bronchoconstriction.

Phytoestrogen compounds are naturally occurring plant hormones showing a structural similarity to 17β-estradiol. Phytoestrogens consist of a number of classes including isoflavones, coumestans, lignans and resorcylic acid lactones. The class of isoflavones consists of among others genistein, daidzein, equol, glycitein, biochanin A, formononetin, and O-desmethylangolesin. The isoflavones genistein and daidzein are found almost uniquely in soybeans. When present in the plant the isoflavones are mainly in a glucoside form, i.e. attached to a sugar molecule. Isoflavones in this glucoside form can be deconjugated to yield isoflavones in a so-called aglycone form, which is the biologically more active form of isoflavones and which is absorbed faster and to a greater extent in the human gut than isoflavones in the glucoside form. *In vitro* studies have examined the relative estrogenic effect exerted by various phytoestrogens including isoflavones. The resulting potencies as compared to estradiol (having a relative potency of 100), have been reported by Knight (Maturitas 22, 167-175 (1995)) for among others genistein (0.084) and daidzein (0.013). However, the results also showed that the estrogen receptor complexes formed by estradiol and isoflavones such as genistein and daidzein are functionally equivalent. The comparative dissociation constant of genistein for the estrogen receptor, as determined in competitive binding assays, was found to be from 100 to 10.000 times higher than that of estradiol.

Soy proteins are involved in a reduction of cholesterol and triglyceride levels, they are easily digestible, and they represent an efficient sole protein source for maintaining the nitrogen balance. Soy isoflavones in high intakes further enhances this effect. Phospholipids, such as soy lecithins, especially soy phosphatidyl choline have been shown to effect total serum cholesterol levels and/or to increase serum HLDL-cholesterol levels. Dietary fibers, such as soybean fibers, especially soy cotyledon

fibers have been shown to lower total serum cholesterol levels, to improve glucose tolerance, to increase insulin sensitivity, to normalize the gastrointestinal function, and to exert no influence on the absorption of essential minerals.

The term "naturally occurring" substance as used in the present specification refers to a substance originally isolated from a natural source, such as an animal or a plant, for example a soy plant, or modified forms of such a substance. The naturally occurring substance for use in a bread according to the present invention may be included in a bread according to the present invention as part of the natural source or in any type of extract, isolate or the like thereof, or it may have been isolated from a plant source or synthesized biologically, microbiologically, or chemically or by any other means.

The terms "exogenously added protein" and "exogenously added dietary fibres" as used throughout the present specification and the appended claims shall be taken to mean protein or dietary fibres which are not part of the flour for use in a method according to the present invention as such, but which ends up in bread by virtue of having been added to or being among or present in the starting materials or the intermediate products in a process for manufacturing said bread, in this case for instance the protein source for use in a method according to the present invention.

The term "protein source" as used throughout the present specification and the appended claims shall be taken to mean a composition comprising protein. For the purpose of the present invention, a protein source may be the source of any number of proteins of any origin. The term itself shall provide no limitations as to the amount of protein present in the protein source and a protein source for use in a method according to the present invention may additionally comprise any number of non-protein components.

DETAILED DESCRIPTION OF THE INVENTION

A bread according to the present invention may be prepared by the addition of all the constituents individually. However according to an especially preferred embodiment of the present invention all soy based constituents are added in a soy blend.

A soy blend of the present invention could comprise soy constituents from any soy source available such as soy flour or any kind of a soy concentrate. In the presently most preferred embodiment of this invention said soy source is a soy protein source

comprising isolated soy protein. In a preferred embodiment of the invention, said soy blend comprises soy protein in the amount of at least 45 weight percent of the total protein content. In a preferred embodiment of the invention the total protein content provides at least 15% of the total energy content of said soy blend. In a preferred embodiment of the invention the ratio of arginine to lysine of said soy protein equals to at least 1.

In a preferred embodiment of the invention the phytoestrogen compound is present in an amount of at least about 0,10 weight percent of the soy protein content of the soy blend. In a most preferred embodiment of the invention the phytoestrogen compound is selected among isoflavones. In a most preferred embodiment of the invention the isoflavones are selected from the group comprising genistein, daidzein, glycitein and equol. In a preferred embodiment of the invention some or all of the isoflavones are present in the aglycone form.

In the most preferred embodiment of the invention the soy blend further comprises soy phospholipids in an amount sufficient to provide at least 15 percent of the total energy contained in the soy blend. In a preferred embodiment of the invention the soy phospholipid source comprises at least 10 weight percent phosphatidylcholine. In a preferred embodiment of the invention the soy phospholipid source is lecithin.

In a preferred embodiment of the invention the soy blend further comprises soy dietary fibre in an amount of at least 5 weight percent. In a preferred embodiment of the invention the weight ratio of soy protein to dietary fibre is at least about 1. In a preferred embodiment of the invention the soy dietary fibre was soy cotyledon fibre.

According to a method of the present invention the soy blend is incorporated into the dough of bread. In another method of the invention the soy blend is incorporated into soy beads prior to being incorporated into dough of bread.

According to the present invention the soy blend is incorporated into dough of bread in amounts appropriate to obtain a content of soy protein corresponding to 5-12 % by weight of the dough. In a preferred embodiment of the invention the soy blend or soy beads further comprise soy dietary fibre and soy phospholipids. The soy blend or soy beads are incorporated into the dough in sufficient amounts to obtain a content of soy

dietary fibre corresponding to 0,9-2,7 % by weight of the dough and a content of soy phospholipids corresponding to 0,07-0,83% by weight of the dough.

Hydrocolloids and proteinaceous materials all have a negative effect on bread quality. Thus, incorporating high amounts of exogenous soy protein into bread will result in reduced eating quality and reduced raising ability compared to traditional bread. This will be even worse in the occasion that the bread further comprises high amounts of an exogenous soy fibre. Furthermore bread comprising soy tends to preserve moisture for longer periods than traditional bread.

It's known that water has to be absorbed by the protein in the wheat flour in order to allow its transformation into gluten. If any absorbent material is added to a formulation of a bread it takes away water from the protein, thereby reducing gluten development with the effect of impairing the quality of the baked bread. Thus, in preferred embodiments of the invention the eating quality and raising ability will be improved by pre-hydrating the soy blend and adjusting the amount of water added to the dough. Use of these techniques allow much higher levels of soy to be incorporated into bread than previously

In a method according to the present invention the soy blend is preferably pre-hydrated prior to being incorporated into dough. The pre-hydration takes place by dispersing the soy blend in water for about 30 seconds or it can take place by simple mixing with water, with or without soaking. The purpose with mixing the soy blend with water alone is to obtain a homogenous mixture. By this pre-hydration the soy protein will be stabilised as a lot of water molecules will be forced around and in between the protein molecules and side chains thereof.

The composition of the soy blend described above is variable. In an embodiment of the invention the soy blend comprises soy protein as the only soy constituent. In a preferred embodiment of the invention the soy blend further comprises soy dietary fibre and soy phospholipids. In this occasion the soy blend is referred to as an Abacor blend. Several variations of Abacor blend exist, thus Abacor Br, Abacor N and Abacor Sw are preferred embodiments of the invention to be explained in detail below. According to the preferred embodiments of the invention Abacor blend comprises 59 - 72% (w/w) soy protein, 12-18% (w/w) soy dietary fibre and 1-5 % (w/w) soy phospholipids.

Abacor Br comprises Soy protein isolate FXP HO 161 IP from Protein Technologies International, MO, USA comprising 87% soy protein, Fibrim 1450 from Protein Technologies International, MO, USA comprising 12% (w/w) soy protein and 80% dietary soy fibre and Epikuron 100SP/130P from Lucas Meyer GmbH & Co., Hamburg, Germany comprising either 20-24% (w/w) or 30-33% (w/w) phosphatidyl choline.

- The original Abacor Br comprises 75,7g Soy protein isolate FXP HO 161 IP, 18,9 g Fibrim 1450 and 5,4g Epikuron 100SP/130P. Thus the original Abacor Br comprises 68 % (w/w) soy protein, 15 % (w/w) soy dietary fibre and at least 1 % (w/w) phosphatidyl choline.
- The improved Abacor Br comprises 64,3g Soy protein isolate FXP HO 161 IP, 21,4 g Fibrim 1450 and 14,3 g Epikuron 130P. Thus the improved Abacor Br comprises 59 % (w/w) soy protein, 17% (w/w) soy dietary fibre and up to 4,7 % (w/w) phosphatidyl choline.

Abacor N comprises Soy protein isolate FXP HO 161 IP comprising 87 % soy protein, Fibrim 1020 from Protein Technologies International, MO, USA comprising 16 % (w/w) soy protein and 64 % dietary soy fibre and Epikuron 100SP/130P comprising either 20-24% (w/w) or 30-33% (w/w) phosphatidyl choline.

- The original Abacor N comprises 75,70g Soy protein isolate FXP HO 161 IP, 18,9 g Fibrim 1020 and 5,4g Epikuron 100SP/130P. Thus the original Abacor N comprises 69 % (w/w) soy protein, 12% (w/w) soy dietary fibre and at least 1 % (w/w) phosphatidyl choline.
- The improved Abacor N comprises 64,3g Soy protein isolate FXP HO 161 IP, 21,4 g Fibrim 1020 and 14,3 g Epikuron 130P. Thus the improved Abacor Br comprises 60 % (w/w) soy protein, 14 % (w/w) soy dietary fibre and maximum 4,7 % (w/w) phosphatidyl choline.

Abacor Sw comprises Soy protein isolate PRO FAM 940 from MultiChem Wallinco, Oslo, Norway comprising 90% soy protein, Fibrim 1020 comprising 16 % (w/w) soy protein and 64 % dietary soy fibre and Epikuron 100SP/130P comprising either 20-24% (w/w) or 30-33% (w/w) phosphatidyl choline.

- The original Abacor Sw comprises 75,7 g Soy protein isolate PRO FAM 940, 18,9 g Fibrim 1020 and 5,4 g Epikuron 100SP/130P. Thus the original Abacor Br comprises 71 % (w/w) soy protein 12 % (w/w) soy dietary fibre and at least 1% (w/w) phosphatidyl choline

- The improved Abacor Sw comprises 64,3 g Soy protein isolate PRO FAM, 21,4g Fibrim 1020 and 14,3 g Epikuron 130P. Thus the improved Abacor Br comprises 61 % (w/w) soy protein, 14 % (w/w) soy dietary fibre and up to 4,7 % (w/w) phosphatidyl choline

As mentioned above incorporating soy blend into dough will influence the dough moisture. Compensation for this is achieved by pre-hydrating the soy-blend or simply by incorporating extra water into dough. As it appears from the above water is the preferred liquid for this use, though any liquid chosen from the group consisting of water, milk and juice could be used for pre-hydrating the soy blend. In the preferred embodiments of the invention the amount of water used for the pre-hydration was between 5 and 17 grams of water for each gram of soy fibre being incorporated into the dough.

Another parameter influencing the moisture is the flour to be incorporated into the dough. According to the present invention any kind of common flour for making bread such as wheat flour, whole meal flour, gluten, rye flour, oat flour and oat flakes can be incorporated into the dough. In order to obtain a proper bread quality it's important to adjust the dough moisture after the kind of flour and the flour quality. Further, in some occasions the bread quality may be improved if e.g. oat flakes and whole meal flour is soaked in water prior to being incorporated into dough.

In preferred embodiments of the invention the English flour "Prarie Gold" and the Norwegian flour "Spesialmel" from "Norgesmøllerne DA", Bergen, Norway are used. The water absorption is higher for the English flour than for the Norwegian flour and there is compensated for this by adding extra water to the dough comprising the English flour. The development time for those two brands of wheat flour is different thus indicating differences in flour quality. Another parameter indicating differences in flour quality is the gluten content and the protein level of the flour e.g. the protein levels differ among the brands Strong Bread Making Flour, Dark Northern Springs flour and Nimrod flour. Exogenous baking additives like gluten, emulsifiers and enzyme additives can compensate for differences in flour quality.

As mentioned above the bread quality will improve significantly when incorporating an appropriate enzyme preparation into the dough. Thus, according to the preferred

embodiments of the invention, exogenous enzyme is incorporated in amounts of 0 or 0,17-3,0% by weight of the fibre content of the dough. Addition of an amymolytic enzyme preparation will increase the volume and induce the formation of a nicer crust and a better breadcrumb structure of the bread. In an embodiment of the invention Xylanase and Fungal Alpha Amylase was shown to improve the volume of the bread. In another embodiment of the invention the enzyme preparation has transglutaminase and hemicellulase activities in addition to the amymolytic enzyme activity. This enzyme preparation is obtained from specific cultures of Aspergillus oryzae and it is available as Veron® CLX from Röhm Enzyme Gmbh.

Enzyme products comprising transglutaminase provide an improved bread quality by stabilising the gluten structure. Transglutaminase creates new bonds between amino acids as it links peptide chains and stabilises the protein structure by catalysing an acyl transfer reaction between the amino acids lysine and glutamine. Gluten is, together with starch and hemicelluloses, responsible for the properties of the dough and influences the entire baking process up to the finished bread. Veron® CLX therefore crosslinks the gluten and improves the consistency of the dough. By using Veron® CLX high baking volume, dry and fluffy dough, good dough stability, good proving stability and good gas retention capacity is obtained. Furthermore, Veron® CLX provides an improved bread quality, a nicer bread crust and a good breadcrumb structure.

The emulsifier usually contained in baking additives are substances promoting the homogeneity of the dough through their surfactant properties. By using an emulsifier the pores in the breadcrumb will render more dense whereby the bread quality will improve. Veron® CLX provides these properties thus reducing the need of emulsifiers. In preferred embodiments of the invention emulsifiers such as Sodium Stearoyl Lactylate (SSL) from Paalgard Industry A/S, Denmark and DATA are incorporated into the dough in amounts corresponding to 9,5 –19 % by weight of the fibre content of the dough.

It appears from the above, that gluten is essential for the bread quality. Accordingly, incorporation of exogenous gluten is essential for good baking results. Thus, the preferred embodiments of the invention comprise incorporation of exogenous gluten, such as Protinax from SFK Norge AS, Skytta, Norway, into dough in amounts

corresponding to 0 or 100-530 % by weight of the fibre content of the dough. Thereby both the dough extensibility and the gas retention capability are enhanced.

In addition to the baking additives mentioned above, fat can be added in order to improve the volume of the bread. In an embodiment of the invention fat is included at a level of 2% corresponding to the amount currently used in normal white bread.

Any kind of yeast could be used for the fermentation. According to a preferred embodiment of the invention instant dry yeast such as Instant dry yeast for lean doughs from Lesaffre, France and Fernipan from Gist-brocades, Netherlands is used. There appears to be no difference in the fermentation rates measured when using these two kinds of instant dry yeast.

According to the invention it's an option to incorporate shortening into dough, though a preferred embodiment of the invention does not comprise use of shortening for making wholemeal bread as it appears to make the breadcrumb divide just below the crust.

In one preferred embodiment of the invention the dough is mixed by use of a commercial dough mixer such as Diosna SP 40 from (Diosna, Dierks & Söhne Gmbh, Osnabruk, Germany). If the soy blend is pre-hydrated at the point of addition the remainder ingredients are mixed with 2 minutes at 100 rpm and 3,5 minutes at 200 rpm. A final mixing programme whereby all the ingredients are mixed to dough consists of 2 minutes at 100 rpm and 0,5 minutes at 200 rpm. If the soy preparation is dry at the point of addition it is sieved together with the flour and the mixing programme is reduced to consist of the final mixing. After finalising the mixing procedure the dough temperature appears to be 25-27°C, in the event that it is measured.

In another embodiment of the invention the dough is mixed by use of a spiral mixer such as a 32kg Spiral Mixer. All the ingredients including the pre-hydrated or dry beads are mixed with 2 minutes at 100 rpm and 12 minutes at 200 rpm.

In another preferred embodiment of the invention the dough is mixed using atmospheric mixing or atmospheric/vacuum mixing such as Tweedy 35. All the ingredients including the pre-hydrated or dry soy preparation are placed in a CBP unit

and dough is mixed for the time required for about 11 watts per kilo to be achieved. If atmospheric/vacuum mixing is used a 15" vacuum is pulled at 48 watt hours.

After mixing, the dough rests for up to 30 minutes. From an embodiment of the invention it appears that resting makes the crust of white flour bread more open with pores that looks like wounds and that no resting gives the highest bread volume. Now the dough is divided into pieces by hand or by use of a machine such as a single stage vacuum divider from Glimek AB, Glimåkra, Sweden. In preferred embodiments of the invention the sizes of the dough pieces are between 460g and 930g. The dough pieces are moulded by hand or by using a machine such as a conical rounder from Glimek AB.

In an embodiment of the invention an intermediate proofing as e.g. 5-10 minutes is applied. In an embodiment of the invention the intermediate prove takes place at a conveyor belt before sheeting, curling and the final mould. In another embodiment of the invention the intermediate proofing takes place in an intermediate proof cabinet.

The dough is finally moulded by hand or by using a machine such as a drum and roller sheeter from Glimek AB or a CCFRA moulder from Sorensen Moulder. Alternatively it is put into rye baskets dusted with rye flour or pans. According to the invention it appears that use of mechanical sheeting gives a smoother bread crust than sheeting the dough by hand. When handling the dough by machine it's essential that the moisture is appropriate. Thus, if the dough is too sticky there might appear a problem with the dough weight as the dough sticks to the rollers in the mechanical roller sheeter. In preferred embodiments of the invention the dough moisture appears to be 43 - 45% in the event it's determined.

The dough proves in a proofing cabinet. In one embodiment of the invention the dough proves for 35 minutes with the cabinet set at 37°C with 78% RH. In another embodiment of the invention the dough proves to touch with the cabinet set at 43°C with 80% R/H.

In one embodiment of the invention the dough proves in rye baskets and the dough is turned upside down onto baking trays. The dough pieces are then placed on steel trays. In another embodiment of the invention the dough pieces are placed in greased 400g tins and proved to a height of 10 cm.

After proofing, the loaves are baked. Any convenient baking time and temperature can be used. In a preferred embodiment of the invention the baking time is between 20 and 35 minutes and the baking temperature is between 220 and 270°C. In an embodiment of the invention steam is added during the first 20-30 seconds of baking or after half the baking time.

From a subjective quality test performed on 19-hour-old bread prepared according to preferred embodiments of the invention and stored at room temperature in plastic bags it appears that bread made according to the present invention has a good eating quality. The test panel consisted of 2 skilled bakers and 2 research scientists, and the results are listed below:

Sensory characteristic	White bread	Whole meal flour bread
Soy flacour	3	4
Bitter flavour	3	4
Powder feeling, clinginess	3	2, the bread piece increase in volume in the mouth
Rancidity	4	4
Smell, odour, wet hay	3	2
Crumb consistency, wetness	2	3

1 is poor quality or the sensory characteristic is very dominating
4 is very good quality or the sensory characteristic is not tasted

The soy protein for use in a bread according to the present invention can be provided by isolated soy protein, soy protein concentrate, soy flour or the like or any combination thereof. Isolated soy protein is preferred. Processed Isolated soy protein is particularly preferred.

Isolated soy protein is the major proteinacious fraction of soybeans. It is prepared from high quality, dehulled, defatted soybeans by removing a preponderance of the non-protein components resulting in an isolated soy protein fraction which in the present context shall contain at least 90 percent protein (N x 6.25) on a moisture free basis. The preparation takes place through a series of steps in which the soybean protein portion is separated from the rest of the soybean. The removal of carbohydrate results in a product, which is essentially bland in flavor and therefore particularly useful in a bread for humans.

Soy protein concentrates are made by removing most of the oil and water-soluble non-protein constituents from defatted and dehulled soybeans. In the present context a soy protein concentrate shall preferably contain at least 65 percent protein on a moisture-free basis.

The soy protein can also be provided by soy flour, which can be full-fat or defatted soy flour. Full-fat soy flour comes from whole, dehulled soybeans that have been ground into a fine powder and, as the name implies, still contains the fat naturally found in soybeans. Defatted soy flour comes from whole, dehulled, defatted soybeans that have been ground into a fine powder. Soy flour is approximately 50 percent soy protein on a dry weight basis in the present context.

The soy protein used in a bread according to the present invention should preferably supply all the essential amino acids in the amounts required for humans. Preferably, the soy protein should also meet or exceed the essential amino acid requirement pattern for children and adults as established by the Food and Agricultural Organization, World Health Organization and United Nations University (FAO/WHO, UNU). Furthermore, the preferred soy protein should be comparable in digestibility to milk, meat, fish, and egg protein. Finally, the preferred soy protein shall be effective in maintaining nitrogen balance when consumed at the recommended protein intake level.

Researcher have shown that specific amino acids may to some extent effect serum lipid levels and potentially alleviate cardiovascular diseases. Animal studies have indicated that the amino acid lysine increases serum cholesterol levels, while arginine counteracts this effect (Kurowska et al., J. Nutr. 124, 364-370 (1994) and Sanchez et al., Med. Hypotheses 35, 324-329 (1991). This observation appears to be in correspondence with the well established influence of NO on vasodilation, since arginine may potentially be converted to citrullin and NO by NO-synthetase. Thus according to a presently preferred hyphothesis soy protein having a high arginine to lysine ratio effects serum lipid levels and alleviates symptoms of cardiovascular diseases to a greater extent than soy protein having a lower or normal arginine to lysine ratio. Consequently, isolated, potentially processed, soy protein having a high arginine to lysine ratio is a particularly preferred soy protein source in a bread according to the present invention. Preferably the soy protein of the soy protein source

in a bread according to the present invention should have an arginine to lysine ratio of at least about 1.0, such as at least about 1.1, for example at least about 1.2, such as at least about 1.3, for example at least about 1.4, such as at least about 1.5, for example at least about 1.6, such as at least about 1.7, for example at least about 1.8, such as at least about 1.9, for example more than about 2, such as at least about 2.1, for example at least about 2.2, such as at least about 2.5, for example at least about 2.75, such as at least about 3, for example more than about 3.3, such as at least about 3.6, for example at least about 4, such as at least about 4.5, for example at least about 5, such as at least about 6, for example at least about 7, such as at least about 8, for example at least about 9, such as at least about 10, for example at least about 11, such as at least about 12, for example at least about 13, such as at least about 14.

Preferred isolated soy protein products meeting some or all of the foregoing requirements are supplied by Protein Technologies International, Inc. under the brand name SUPRO®. SUPRO® isolated soy proteins are supplied in many different qualities and SUPRO® XT 12C is one particularly preferred quality. The currently most preferred quality is termed SUPRO® FXP-HO159.

The soy protein is preferably the main protein source in a bread according to the present invention. However, parts of the protein source may be provided by other proteins such as e.g. skimmed milk, preferably as a powder, and other vegetable or animal proteins including diary proteins.

In a preferred embodiment of the invention the soy protein is provided by isolated soy protein

Phytoestrogen compounds according to the present invention are defined as naturally occurring plant substances, said substances being either structurally or functionally similar to 17□-estradiol or generating estrogenic effects. Phytoestrogens consist of a number of classes including isoflavones, coumestans, lignans and resorcylic acid lactones. Examples of isoflavones according to the present invention are genistein, daidzein, equol, glycitein, biochanin A, formononetin, and O-desmethylangolesin. The phytoestrogen compounds of a bread according to the present invention are preferably isoflavones, more preferably genistein, daidzein, glycitein and/or equol, yet more preferably genistein and/or daidzein, and even more preferably genistein. A preferred bread according to the present invention may accordingly comprise a single

isoflavone, such as genistein, daidzein, glycitein or equol, or it may comprise at least one isoflavone selected from the group comprising at least genistein, daidzein, glycitein and equol. When present in the plant the isoflavones are mainly in a glucoside form, i.e. attached to a sugar molecule. This glucoside form can be deconjugated to yield a so-called aglycone form, which is the biologically active species. A bread according to the present invention may comprise isoflavones in glucoside and/or aglycone forms regardless of whether the deconjugation to the aglycone form has taken place biologically, in vitro or by any other means whereby the isoflavones are included in a bread according to the present invention or if the aglycone forms are the native form of the isoflavones.

The phytoestrogen compound is preferably present in an amount of at least about 0.10 weight percent of the soy protein content. More preferably the phytoestrogen compound is present in an amount of at least 0.10 weight percent of the soy protein content, such as at least about 0.11 weight percent, for example at least about 0.12 weight percent, such as at least about 0.14 weight percent, for example at least about 0.16 weight percent, such as at least about 0.18 weight percent, for example at least about 0.20 weight percent, such as at least about 0.22 weight percent, for example at least about 0.24 weight percent, such as at least about 0.25 weight percent, for example more than about 0.25 weight percent, such as at least about 0.26 weight percent, for example at least about 0.28 weight percent, such as at least about 0.30 weight percent, for example at least about 0.32 weight percent, such as at least about 0.33 weight percent, for example more than about 0.33 weight percent, such as at least about 0.35 weight percent, for example at least about 0.40 weight percent, such as at least about 0.45 weight percent, for example at least about 0.50 weight percent, such as at least about 0.55 weight percent, for example at least about 0.60 weight percent, such as at least about 0.65 weight percent, for example at least about 0.70 weight percent, such as at least about 0.75 weight percent, for example at least about 0.80 weight percent, such as at least about 0.85 weight percent, for example at least about 0.90 weight percent, such as at least about 1.0 weight percent of the soy protein content, and preferably less than 2.50 weight percent of the soy protein content.

In the past, the downstream processing techniques used in the preparation of soy proteins have included steps that removed and/or destroyed isoflavones. Methods are available today, which provide soy protein products with high, fixed levels of naturally occurring isoflavones. The isoflavones according to the present invention in glucoside

and/or aglycone forms can be included in a bread according to the present invention as part of such soy protein products and/or by themselves and/or as part of any other bread comprising isoflavones.

According to a preferred embodiment of the present invention the soy protein used in a process for the preparation of a bread according to the present invention retain the ability to lower blood cholesterol levels upon ingestion, characterised by having more than a further specified minimum content of intact 7S (α + α' + β) and/or 11S subunits (A + B).

Without wishing to be bound by theory it is believed that ingestion of soy proteins with minimum content of intact 7S (α + α' + β) and/or 11S subunits (A + B) facilitate the formation of breakdown products in the digestive tract, in the form of peptides which elicit an effect in the form of cholesterol lowering.

The present invention also provides the use of soy protein in a bread according to the present invention, which retains the ability to lower blood cholesterol levels upon ingestion, characterised by minimum content of intact 7S (α + α' + β) and/or 11S subunits (A + B), and also provides for the use of such a bread in which the soy protein retains the ability to lower blood cholesterol levels upon ingestion to obtain a health benefit.

In one aspect the present invention provides bread comprising soy protein, which retains the ability to lower blood cholesterol levels upon ingestion, characterised by a minimum content of intact 7S (α + α' + β) and/or 11S subunits (A + B). The soy protein of such a bread more specifically are believed facilitate the formation of peptides which has a cholesterol lowering effect.

In another aspect, the present invention provides a method for manufacturing a bread product, wherein the soy protein retains the ability to lower blood cholesterol levels upon ingestion and characterised by a minimum content of intact 7S (α + α' + β) and/or 11S subunits (A + B)

The amount of intact 7S subunits (α + α' + β) and 11S subunits (A + B) in the soy protein used in the preparation of a bread according to the invention preferably constitute more than 5 % of the total soy protein content, such as more than 10 %, for

example more than 15 %, , such as more than 20 %, for example more than 25 %, such as more than 30 %, for example more than 31 %, such as more than 32 %, for example more than 33 %, such as more than 34 %, for example more than 35 %, such as more than 36 %, for example more than 37 %, such as more than 38 %, for example more than 39 %, such as more than 40 %, for example more than 41 %, such as more than 42 %, for example more than 43 %, such as more than 44 %, for example more than 45 %, such as more than 46 %, for example more than 47 %, such as more than 48 %, for example more than 49 %, such as more than 50 %, for example more than 51 %, such as more than 52 %, for example more than 53 %, such as more than 54 %, for example more than 55 %, such as more than 56 %, for example more than 57 %, such as more than 58 %, for example more than 59 %, such as more than 60 %, for example more than 61 %, such as more than 62 %, for example more than 63 %, such as more than 64 %, for example more than 65 %, such as more than 66 %, for example more than 67 %, such as more than 68 %, for example more than 69 %, such as more than 70 %, for example more than 71 %, such as more than 72 %, for example more than 73 %, such as more than 74 %, for example more than 75 %, such as more than 77 %, for example more than 79 %, such as more than 81 %, for example more than 83 %, such as more than 85 %, for example more than 90 %, such as more than 95 %.

The amount of intact 7S subunits (α + α' + β) in the soy protein used in the preparation of a bread according to the invention preferably constitute more than 1 % of the total soy protein content, such as more than 2 %, for example more than 3 %, such asmore than 4 %, for example more than 5 %, such as more than 6 %, for example more than 7 %, such as more than 8 %, for example more than 9 %, such as more than 10 %, for example more than 11 %, such as more than 12 %, for example more than 13 %, such as more than 14 %, for example more than 15 %, such as more than 16 %, for example more than 17 %, such as more than 18 %, for example more than 19 %, such as more than 20 %, for example more than 21 %, such as more than 22 %, for example more than 23 %, such as more than 24 %, for example more than 25 %, such as more than 26 %, for example more than 27 %, such as more than 28 %, for example more than 29 %, such as more than 30 %, for example more than 31 %, such as more than 32 %, for example more than 33 %, such as more than 34 %, for example more than 35 %, such as more than 36 %, for example more than 37 %, such as more than 38 %, for example more than 39 %, such as more than 40 %, for example more than 41 %, such as more than 42 %, for example more than 43 %, such

as more than 44 %, for example more than 45 %, such as more than 46 %, for example more than 47 %, such as more than 48 %, for example more than 49 %, such as more than 50 %, for example more than 55 %, such as more than 60 %, for example more than 65 %, such as more than 70 %, for example more than 75 %, such as more than 80 %, such as more than 85 %, for example more than 90 %, such as more than 95 %.

The amount of intact 7S subunit α in the soy protein used in the preparation of a bread according to the invention preferably constitute more than 1 % of the total soy protein content, such as more than 2 %, for example more than 3 %, such asmore than 4 %, for example more than 5 %, such as more than 6 %, for example more than 7 %, such as more than 8 %, for example more than 9 %, such as more than 10 %, for example more than 11 %, such as more than 12 %, for example more than 13 %, such as more than 14 %, for example more than 15 %, such as more than 16 %, for example more than 17 %, such as more than 18 %, for example more than 19 %, such as more than 20 %, for example more than 21 %, such as more than 22 %, for example more than 23 %, such as more than 24 %, for example more than 25 %, such as more than 26 %, for example more than 27 %, such as more than 28 %, for example more than 29 %, such as more than 30 %, for example more than 31 %, such as more than 32 %, for example more than 33 %, such as more than 34 %, for example more than 35 %, such as more than 36 %, for example more than 37 %, such as more than 38 %, for example more than 39 %, such as more than 40 %, for example more than 41 %, such as more than 42 %, for example more than 43 %, such as more than 44 %, for example more than 45 %, such as more than 46 %, for example more than 47 %, such as more than 48 %, for example more than 49 %, such as more than 50 %, for example more than 55 %, such as more than 60 %, for example more than 65 %, such as more than 70 %, for example more than 75 %, such as more than 80 %, such as more than 85 %, for example more than 90 %, such as more than 95 %.

The amount of intact 7S subunit α' in the soy protein used in the preparation of a bread according to the invention preferably constitute more than 1 % of the total soy protein content, such as more than 2 %, for example more than 3 %, such asmore than 4 %, for example more than 5 %, such as more than 6 %, for example more than 7 %, such as more than 8 %, for example more than 9 %, such as more than 10 %, for example more than 11 %, such as more than 12 %, for example more than 13 %, such as more than 14 %, for example more than 15 %, such as more than 16 %, for example more

than 17 %, such as more than 18 %, for example more than 19 %, such as more than 20 %, for example more than 21 %, such as more than 22 %, for example more than 23 %, such as more than 24 %, for example more than 25 %, such as more than 26 %, for example more than 27 %, such as more than 28 %, for example more than 29 %, such as more than 30 %, for example more than 31 %, such as more than 32 %, for example more than 33 %, such as more than 34 %, for example more than 35 %, such as more than 36 %, for example more than 37 %, such as more than 38 %, for example more than 39 %, such as more than 40 %, for example more than 41 %, such as more than 42 %, for example more than 43 %, such as more than 44 %, for example more than 45 %, such as more than 46 %, for example more than 47 %, such as more than 48 %, for example more than 49 %, such as more than 50 %, for example more than 55 %, such as more than 60 %, for example more than 65 %, such as more than 70 %, for example more than 75 %, such as more than 80 %, such as more than 85 %, for example more than 90 %, such as more than 95 %.

According to a particularly preferred embodiment of the present invention the soy protein used in the preparation of a bread according to the invention also contain phytoestrogens, such as isoflavons. The phytoestrogen compound is preferably present in an amount of at least about 0.12 weight percent of the soy protein content, such as at least about 0.14 weight percent, for example at least about 0.16 weight percent, such as at least about 0.18 weight percent, for example at least about 0.20 weight percent, such as at least about 0.22 weight percent, for example at least about 0.24 weight percent, such as at least about 0.25 weight percent, for example more than about 0.25 weight percent, such as at least about 0.26 weight percent, for example at least about 0.28 weight percent, such as at least about 0.30 weight percent, for example at least about 0.32 weight percent, such as at least about 0.33 weight percent, for example more than about 0.33 weight percent, such as at least about 0.35 weight percent, for example at least about 0.40 weight percent, such as at least about 0.45 weight percent, for example at least about 0.50 weight percent, such as at least about 0.55 weight percent, for example at least about 0.60 weight percent, such as at least about 0.65 weight percent, for example at least about 0.70 weight percent, such as at least about 0.75 weight percent, for example at least about 0.80 weight percent, such as at least about 0.85 weight percent, for example at least about 0.90 weight percent, such as at least about 1.0 weight percent of the soy protein content, and preferably less than 2.50 weight percent of the soy protein content.

In soy protein used in the preparation of a bread according to the invention with an amount of isoflavones of at least 0.16 weight % of the soy protein, the amount of intact 7S subunits (α + α' + β) and 11S subunits (A + B) preferably constitute more 5 % of the total soy protein content, such as more than 10 %, for example more than 15 %, , such as more than 20 %, for example more than 25 %, such as more than 30 %, for example more than 31 %, such as more than 32 %, for example more than 33 %, such as more than 34 %, for example more than 35 %, such as more than 36 %, for example more than 37 %, such as more than 38 %, for example more than 39 %, such as more than 40 %, for example more than 41 %, such as more than 42 %, for example more than 43 %, such as more than 44 %, for example more than 45 %, such as more than 46 %, for example more than 47 %, such as more than 48 %, for example more than 49 %, such as more than 50 %, for example more than 51 %, such as more than 52 %, for example more than 53 %, such as more than 54 %, for example more than 55 %, such as more than 56 %, for example more than 57 %, such as more than 58 %, for example more than 59 %, such as more than 60 %, for example more than 61 %, such as more than 62 %, for example more than 63 %, such as more than 64 %, for example more than 65 %, such as more than 66 %, for example more than 67 %, such as more than 68 %, for example more than 69 %, such as more than 70 %, for example more than 71 %, such as more than 72 %, for example more than 73 %, such as more than 74 %, for example more than 75 %, such as more than 77 %, for example more than 79 %, such as more than 81 %, for example more than 83 %, such as more than 85 %, for example more than 90 %, such as more than 95 %.

In soy protein used in the preparation of a bread according to the invention with an amount of isoflavones of at least 0.16 %, the amount of intact 7S subunits (α + α' + β) preferably constitute more than than 3 %, such as more than 4 %, for example more than 5 %, such as more than 6 %, for example more than 7 %, such as more than 8 %, for example more than 9 %, such as more than 10 %, for example more than 11 %, such as more than 12 %, for example more than 13 %, such as more than 14 %, for example more than 15 %, such as more than 16 %, for example more than 17 %, such as more than 18 %, for example more than 19 %, such as more than 20 %, for example more than 21 %, such as more than 22 %, for example more than 23 %, such as more than 24 %, for example more than 25 %, such as more than 26 %, for example more than 27 %, such as more than 28 %, for example more than 29 %, such as more than 30 %, for example more than 31 %, such as more than 32 %, for

example more than 33 %, such as more than 34 %, for example more than 35 %, such as more than 36 %, for example more than 37 %, such as more than 38 %, for example more than 39 %, such as more than 40 %, for example more than 41 %, such as more than 42 %, for example more than 43 %, such as more than 44 %, for example more than 45 %, such as more than 46 %, for example more than 47 %, such as more than 48 %, for example more than 49 %, such as more than 50 %, for example more than 55 %, such as more than 60 %, for example more than 65 %, such as more than 70 %, for example more than 75 %, such as more than 80 %, such as more than 85 %, for example more than 90 %, such as more than 95 %.

In soy protein used in the preparation of a bread according to the invention with an amount of isoflavones of at least 0.16 % of the soy protein, the amount of intact 7S subunit α preferably constitute more than 1 % of the total soy protein content, such as more than 2 %, for example more than 3 %, such asmore than 4 %, for example more than 5 %, such as more than 6 %, for example more than 7 %, such as more than 8 %, for example more than 9 %, such as more than 10 %, for example more than 11 %, such as more than 12 %, for example more than 13 %, such as more than 14 %, for example more than 15 %, such as more than 16 %, for example more than 17 %, such as more than 18 %, for example more than 19 %, such as more than 20 %, for example more than 21 %, such as more than 22 %, for example more than 23 %, such as more than 24 %, for example more than 25 %, such as more than 26 %, for example more than 27 %, such as more than 28 %, for example more than 29 %, such as more than 30 %, for example more than 31 %, such as more than 32 %, for example more than 33 %, such as more than 34 %, for example more than 35 %, such as more than 36 %, for example more than 37 %, such as more than 38 %, for example more than 39 %, such as more than 40 %, for example more than 41 %, such as more than 42 %, for example more than 43 %, such as more than 44 %, for example more than 45 %, such as more than 46 %, for example more than 47 %, such as more than 48 %, for example more than 49 %, such as more than 50 %, for example more than 55 %, such as more than 60 %, for example more than 65 %, such as more than 70 %, for example more than 75 %, such as more than 80 %, such as more than 85 %, for example more than 90 %, such as more than 95 %.

In soy protein used in the preparation of a bread according to the invention with an amount of isoflavones of at least 0.16 % of the soy protein, the amount of intact 7S subunit α' preferably constitute more than 1 % of the total soy protein content, such as

more than 2 %, for example more than 3 %, such asmore than 4 %, for example more than 5 %, such as more than 6 %, for example more than 7 %, such as more than 8 %, for example more than 9 %, such as more than 10 %, for example more than 11 %, such as more than 12 %, for example more than 13 %, such as more than 14 %, for example more than 15 %, such as more than 16 %, for example more than 17 %, such as more than 18 %, for example more than 19 %, such as more than 20 %, for example more than 21 %, such as more than 22 %, for example more than 23 %, such as more than 24 %, for example more than 25 %, such as more than 26 %, for example more than 27 %, such as more than 28 %, for example more than 29 %, such as more than 30 %, for example more than 31 %, such as more than 32 %, for example more than 33 %, such as more than 34 %, for example more than 35 %, such as more than 36 %, for example more than 37 %, such as more than 38 %, for example more than 39 %, such as more than 40 %, for example more than 41 %, such as more than 42 %, for example more than 43 %, such as more than 44 %, for example more than 45 %, such as more than 46 %, for example more than 47 %, such as more than 48 %, for example more than 49 %, such as more than 50 %, for example more than 55 %, such as more than 60 %, for example more than 65 %, such as more than 70 %, for example more than 75 %, such as more than 80 %, such as more than 85 %, for example more than 90 %, such as more than 95 %.

Optionally a soy protein product used in the preparation of a bread according to the invention may have the trypsin inhibitors partly or fully destroyed or removed. The amount of ACTIVE trypsin inhibitor in a soy protein product used in the preparation of a bread according to the invention may preferably be less than 50% of the amount in the original soy bean, such as less than 40%, for example less than 30%, such as less than 25%, for example less than 20%, such as less than 15%, for example less than 10%, such as less than 5 %, for example less than 1%.

The amount of intact 7S subunits (α + α' + β) and 11S subunits (A + B) in the soy protein in a bread according to the invention preferably constitute more than 5 % of the total soy protein content, such as more than 10 %, for example more than 15 %, , such as more than 20 %, for example more than 25 %, such as more than 30 %, for example more than 31 %, such as more than 32 %, for example more than 33 %, such as more than 34 %, for example more than 35 %, such as more than 36 %, for example more than 37 %, such as more than 38 %, for example more than 39 %, such as more than 40 %, for example more than 41 %, such as more than 42 %, for

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The amount of intact 7S subunits (α + α' + β) in the soy protein in a bread according to the invention preferably constitute more than 1 % of the total soy protein content, such as more than 2 %, for example more than 3 %, such asmore than 4 %, for example more than 5 %, such as more than 6 %, for example more than 7 %, such as more than 8 %, for example more than 9 %, such as more than 10 %, for example more than 11 %, such as more than 12 %, for example more than 13 %, such as more than 14 %, for example more than 15 %, such as more than 16 %, for example more than 17 %, such as more than 18 %, for example more than 19 %, such as more than 20 %, for example more than 21 %, such as more than 22 %, for example more than 23 %, such as more than 24 %, for example more than 25 %, such as more than 26 %, for example more than 27 %, such as more than 28 %, for example more than 29 %, such as more than 30 %, for example more than 31 %, such as more than 32 %, for example more than 33 %, such as more than 34 %, for example more than 35 %, such as more than 36 %, for example more than 37 %, such as more than 38 %, for example more than 39 %, such as more than 40 %, for example more than 41 %, such as more than 42 %, for example more than 43 %, such as more than 44 %, for example more than 45 %, such as more than 46 %, for example more than 47 %, such as more than 48 %, for example more than 49 %, such as more than 50 %, for example more than 55 %, such as more than 60 %, for example more than 65 %, such as more than 70 %, for example more than 75 %, such as more than 80 %, such as more than 85 %, for example more than 90 %, such as more than 95 %.

The amount of intact 7S subunit α in the soy protein in a bread according to the invention preferably constitute more than 1 % of the total soy protein content, such as more than 2 %, for example more than 3 %, such asmore than 4 %, for example more than 5 %, such as more than 6 %, for example more than 7 %, such as more than 8 %, for example more than 9 %, such as more than 10 %, for example more than 11 %, such as more than 12 %, for example more than 13 %, such as more than 14 %, for example more than 15 %, such as more than 16 %, for example more than 17 %, such as more than 18 %, for example more than 19 %, such as more than 20 %, for example more than 21 %, such as more than 22 %, for example more than 23 %, such as more than 24 %, for example more than 25 %, such as more than 26 %, for example more than 27 %, such as more than 28 %, for example more than 29 %, such as more than 30 %, for example more than 31 %, such as more than 32 %, for example more than 33 %, such as more than 34 %, for example more than 35 %, such as more than 36 %, for example more than 37 %, such as more than 38 %, for example more than 39 %, such as more than 40 %, for example more than 41 %, such as more than 42 %, for example more than 43 %, such as more than 44 %, for example more than 45 %, such as more than 46 %, for example more than 47 %, such as more than 48 %, for example more than 49 %, such as more than 50 %, for example more than 55 %, such as more than 60 %, for example more than 65 %, such as more than 70 %, for example more than 75 %, such as more than 80 %, such as more than 85 %, for example more than 90 %, such as more than 95 %.

The amount of intact 7S subunit α' in the soy protein in a bread according to the invention preferably constitute more than 1 % of the total soy protein content, such as more than 2 %, for example more than 3 %, such asmore than 4 %, for example more than 5 %, such as more than 6 %, for example more than 7 %, such as more than 8 %, for example more than 9 %, such as more than 10 %, for example more than 11 %, such as more than 12 %, for example more than 13 %, such as more than 14 %, for example more than 15 %, such as more than 16 %, for example more than 17 %, such as more than 18 %, for example more than 19 %, such as more than 20 %, for example more than 21 %, such as more than 22 %, for example more than 23 %, such as more than 24 %, for example more than 25 %, such as more than 26 %, for example more than 27 %, such as more than 28 %, for example more than 29 %, such as more than 30 %, for example more than 31 %, such as more than 32 %, for example more than 33 %, such as more than 34 %, for example more than 35 %, such

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According to a particularly preferred embodiment of the present invention the soy protein in a bread according to the invention also contain phytoestrogens, such as isoflavons. The phytoestrogen compound is preferably present in an amount of at least about 0.12 weight percent of the soy protein content, such as at least about 0.14 weight percent, for example at least about 0.16 weight percent, such as at least about 0.18 weight percent, for example at least about 0.20 weight percent, such as at least about 0.22 weight percent, for example at least about 0.24 weight percent, such as at least about 0.25 weight percent, for example more than about 0.25 weight percent, such as at least about 0.26 weight percent, for example at least about 0.28 weight percent, such as at least about 0.30 weight percent, for example at least about 0.32 weight percent, such as at least about 0.33 weight percent, for example more than about 0.33 weight percent, such as at least about 0.35 weight percent, for example at least about 0.40 weight percent, such as at least about 0.45 weight percent, for example at least about 0.50 weight percent, such as at least about 0.55 weight percent, for example at least about 0.60 weight percent, such as at least about 0.65 weight percent, for example at least about 0.70 weight percent, such as at least about 0.75 weight percent, for example at least about 0.80 weight percent, such as at least about 0.85 weight percent, for example at least about 0.90 weight percent, such as at least about 1.0 weight percent of the soy protein content, and preferably less than 2.50 weight percent of the soy protein content.

In soy protein in a bread according to the invention with an amount of isoflavones of at least 0.16 weight % of the soy protein, the amount of intact 7S subunits ($\alpha + \alpha' + \beta$) and 11S subunits (A + B) preferably constitute more 5 % of the total soy protein content, such as more than 10 %, for example more than 15 %, , such as more than 20 %, for example more than 25 %, such as more than 30 %, for example more than 31 %, such as more than 32 %, for example more than 33 %, such as more than 34 %,

for example more than 35 %, such as more than 36 %, for example more than 37 %, such as more than 38 %, for example more than 39 %, such as more than 40 %, for example more than 41 %, such as more than 42 %, for example more than 43 %, such as more than 44 %, for example more than 45 %, such as more than 46 %, for example more than 47 %, such as more than 48 %, for example more than 49 %, such as more than 50 %, for example more than 51 %, such as more than 52 %, for example more than 53 %, such as more than 54 %, for example more than 55 %, such as more than 56 %, for example more than 57 %, such as more than 58 %, for example more than 59 %, such as more than 60 %, for example more than 61 %, such as more than 62 %, for example more than 63 %, such as more than 64 %, for example more than 65 %, such as more than 66 %, for example more than 67 %, such as more than 68 %, for example more than 69 %, such as more than 70 %, for example more than 71 %, such as more than 72 %, for example more than 73 %, such as more than 74 %, for example more than 75 %, such as more than 77 %, for example more than 79 %, such as more than 81 %, for example more than 83 %, such as more than 85 %, for example more than 90 %, such as more than 95 %.

In soy protein in a bread according to the invention with an amount of isoflavones of at least 0.16 %, the amount of intact 7S subunits ($\alpha + \alpha' + \beta$) preferably constitute more than than 3 %, such as more than 4 %, for example more than 5 %, such as more than 6 %, for example more than 7 %, such as more than 8 %, for example more than 9 %, such as more than 10 %, for example more than 11 %, such as more than 12 %, for example more than 13 %, such as more than 14 %, for example more than 15 %, such as more than 16 %, for example more than 17 %, such as more than 18 %, for example more than 19 %, such as more than 20 %, for example more than 21 %, such as more than 22 %, for example more than 23 %, such as more than 24 %, for example more than 25 %, such as more than 26 %, for example more than 27 %, such as more than 28 %, for example more than 29 %, such as more than 30 %, for example more than 31 %, such as more than 32 %, for example more than 33 %, such as more than 34 %, for example more than 35 %, such as more than 36 %, for example more than 37 %, such as more than 38 %, for example more than 39 %, such as more than 40 %, for example more than 41 %, such as more than 42 %, for example more than 43 %, such as more than 44 %, for example more than 45 %, such as more than 46 %, for example more than 47 %, such as more than 48 %, for example more than 49 %, such as more than 50 %, for example more than 55 %, such as more than 60 %, for example more than 65 %, such as more than 70 %, for

example more than 75 %, such as more than 80 %, such as more than 85 %, for example more than 90 %, such as more than 95 %.

In soy protein in a bread according to the invention with an amount of isoflavones of at least 0.16 % of the soy protein, the amount of intact 7S subunit α preferably constitute more than 1 % of the total soy protein content, such as more than 2 %, for example more than 3 %, such asmore than 4 %, for example more than 5 %, such as more than 6 %, for example more than 7 %, such as more than 8 %, for example more than 9 %, such as more than 10 %, for example more than 11 %, such as more than 12 %, for example more than 13 %, such as more than 14 %, for example more than 15 %, such as more than 16 %, for example more than 17 %, such as more than 18 %, for example more than 19 %, such as more than 20 %, for example more than 21 %, such as more than 22 %, for example more than 23 %, such as more than 24 %, for example more than 25 %, such as more than 26 %, for example more than 27 %, such as more than 28 %, for example more than 29 %, such as more than 30 %, for example more than 31 %, such as more than 32 %, for example more than 33 %, such as more than 34 %, for example more than 35 %, such as more than 36 %, for example more than 37 %, such as more than 38 %, for example more than 39 %, such as more than 40 %, for example more than 41 %, such as more than 42 %, for example more than 43 %, such as more than 44 %, for example more than 45 %, such as more than 46 %, for example more than 47 %, such as more than 48 %, for example more than 49 %, such as more than 50 %, for example more than 55 %, such as more than 60 %, for example more than 65 %, such as more than 70 %, for example more than 75 %, such as more than 80 %, such as more than 85 %, for example more than 90 %, such as more than 95 %.

In soy protein in a bread according to the invention with an amount of isoflavones of at least 0.16 % of the soy protein, the amount of intact 7S subunit α' preferably constitute more than 1 % of the total soy protein content, such as more than 2 %, for example more than 3 %, such asmore than 4 %, for example more than 5 %, such as more than 6 %, for example more than 7 %, such as more than 8 %, for example more than 9 %, such as more than 10 %, for example more than 11 %, such as more than 12 %, for example more than 13 %, such as more than 14 %, for example more than 15 %, such as more than 16 %, for example more than 17 %, such as more than 18 %, for example more than 19 %, such as more than 20 %, for example more than 21 %, such as more than 22 %, for example more than 23 %, such as more than 24 %, for

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The phospholipid source according to the present invention will preferably comprise polyunsaturated fatty acids and monounsaturated fatty acids and optionally also saturated fatty acids. Soy lecithins and □-linolenic acid are particularly preferred. The phospholipid source will preferably comprise at least about 5% phosphatidyl choline, such as at least 10% phosphatidyl choline. The phospholipid source will more preferably comprise at least about 20% phosphatidyl choline, such as at least about 30% phosphatidyl choline, for example at least about 35% phosphatidyl choline, such as at least about 40% phosphatidyl choline, for example at least about 45% phosphatidyl choline, such as at least about 50% phosphatidyl choline, for example more than about 55% phosphatidyl choline phosphatidyl choline by weight, such as at least 60% phosphatidyl choline, for example at least about 65% phosphatidyl choline, such as at least about 70% phosphatidyl choline, for example at least about 71% phosphatidyl choline, such as at least about 72% phosphatidyl choline, for example at least about 73% phosphatidyl choline, such as at least about 74% phosphatidyl choline, for example more than about 75% phosphatidyl choline, such as at least about 76% phosphatidyl choline, for example at least about 77% phosphatidyl choline, such as at least about 78% phosphatidyl choline, for example at least about 79% phosphatidyl choline, for example more than about 80% phosphatidyl choline, such as at least about 85% phosphatidyl choline, for example at least about 90% phosphatidyl choline, such as at least about 98% phosphatidyl choline, for example 100% phosphatidyl choline by weight.

The phospholipid source will preferably comprise polyunsaturated fatty acids and monounsaturated fatty acids and optionally also saturated fatty acids. The amount of polyunsaturated fatty acids and monounsaturated fatty acids, including the essential fatty acids, may range from 35 to 50, preferably 38 to 44, weight percent of the total amount of the fat source. The essential fatty acids are also called omega-6 and omega-3 fatty acids and include linolic acid and/or linolenic acid (□-linolenic acid). The amount of saturated fatty acids may be from 20 to 30 weight percent, preferably 22 to 26 weight percent, of the total amount of the phospholipid source. In a bread according to the present invention, the phospholipid source usually provides from 5 to 70 percent, preferably 10 to 60 percent, such as from 15 to 50 percent, for example from 20 to 40 percent, such as from 25 to 35 percent of the total energy content of the bread.

The phospholipid source preferably provides at least about 5 percent of the total energy content of the bread, such as at least about 10 percent, for example at least about 15 percent, such as at least about 20 percent, for example at least about 21 percent, such as at least about 22 percent, for example at least about 23 percent, such as at least about 24 percent, for example more than about 25 percent, such as at least about 26 percent, for example at least about 27 percent, such as at least about 28 percent, for example at least about 29 percent, such as at least about 30 percent, for example more than about 31 percent, such as at least about 32 percent, for example at least about 33 percent, such as at least about 34 percent, for example at least about 35 percent, such as at least about 36 percent, for example at least about 37 percent, such as at least about 38 percent, for example at least about 39 percent, such as at least about 40 percent, for example at least about 45 percent, such as at least about 50 percent, for example at least about 55 percent, such as at least about 60 percent, for example at least about 65 percent of the total energy content of the bread, and preferably less than 70 percent of the total energy content of the bread.

Preferred phospholipid sources are lecithins and even more preferably soy lecithin. Currently preferred lecithin products are manufactured by SKW Nature Products, BioActives, Freising, Germany are marketed under the brand name of Epikuron 100®, Epikuron 130®.

The dietary fibers used in a presently preferred embodiment of the present invention should preferably comprise a mixture of insoluble fibers and water-soluble fibers also

referred to as soluble fibers. Soluble fibers have a lowering effect on blood cholesterol levels. Examples of dietary fibers comprising soluble fibers are fibers from apples, bananas, oranges, carrots, oats, and soybeans. The dietary fibers preferably comprise soluble fibers in an amount of about 5 weight percent, such as about 10 weight percent, for example about 15 weight percent, such as about 20 weight percent, for example about 25 weight percent, such as about 30 weight percent, for example about 35 weight percent, such as about 40 weight percent, for example about 45 weight percent, such as about 50 weight percent, for example about 55 weight percent, such as about 60 weight percent, for example about 65 weight percent, such as about 70 weight percent, for example about 75 weight percent, such as about 80 weight percent, for example about 85 weight percent, such as about 90 weight percent, for example about 95 weight percent. The dietary fibers used in the present invention are preferably soybean fibers, more preferably soy cotyledon fibers. Such fibers are derived from dehulled and defatted soybean cotyledon and are comprised of a mixture of soluble and insoluble fibers. Soy cotyledon fibers are distinctly different from soybean fibers derived from soy hulls as well as other fiber sources. Soy cotyledon fibers are bland tasting, contain no cholesterol, are low in fat and sodium, and they have good water-binding properties and low caloric content.

Soy cotyledon fibers supplied in a fat-modified and low-cholesterol diet are known to further reduce serum cholesterol levels in a subject suffering from mild to severe hypercholesterolemia. The effect is a lowering of the serum levels of total cholesterol including a lowering of the serum levels of LDL-cholesterol. However, HDL-cholesterol and total triglycerides are not significantly affected by soy cotyledon fibers. Soybean fibers, in particular soy cotyledon fibers, are believed to provide a synergistic effect in combination with soy protein and/or with a phytoestrogen compound, such as naturally occurring isoflavones, or to exert a potentiating effect on the soy protein and/or the phytoestrogen compound, said synergistic or potentiating effect being effective in lowering serum levels of lipid and cholesterol in subjects having normal as well as elevated serum levels of total cholesterol and total triglycerides.

Without wishing to be bound by any specific theory it is presently believed that both soluble dietary fibers (working as nutrients) and insoluble dietary fibers (working as bulking agents), in particular from soybean fibers, more particularly soy cotyledon fibers, provide favorable growth conditions for the microflora in the human gut, which makes the microflora more effective in deconjugating isoflavones in the glucoside form

to the aglycone form. Isoflavones in the aglycone form are absorbed faster and to a greater extent in the human gut than isoflavones in the glucoside form, and isoflavones in the aglycone form are the biologically more active species in the present context. In view hereof it can be understood that administration of a combination of soy proteins, a high, fixed level of isoflavones and a combination of soluble and insoluble fibers may be effective in providing an increased uptake of isoflavones.

Furthermore, again without wishing to be bound by any specific theory, it is presently believed that both soluble dietary fibers (working as nutrients) and insoluble dietary fibers (working as bulking agents), in particular from soybean fibers, more particularly soy cotyledon fibers, provide favorable growth conditions for the microflora in the human gut, which makes the microflora more effective in converting phosphatidyl serine and phosphatidyl ethanolamine into phosphatidyl choline. This capability to decarboxylate phosphatidyl serine into phosphatidyl ethanolamine by the action of pyridoxal phosphate enzymes and further methylate phosphatidyl ethanolamine into phosphatidyl choline has presently only been proven for bacteria. Phosphatidyl choline are absorbed faster and to a greater extent in the human gut than phosphatidyl serine and phosphatidyl ethanolamine, and phosphatidyl choline is the biologically more active species in the present context. In view hereof it can be understood that administration of a combination of soy proteins, a phospholipid source having a high fixed level of phosphoglycerides and a combination of soluble and insoluble fibers may be effective in providing increased levels of phosphatidyl choline from a given phospholipid source and hence provide an increased uptake of phosphatidyl choline from a given phospholipid source.

The amount of dietary fibers of the total weight of a bread according to the present invention on a dry basis is preferably more than 2 weight percent, for example at least 4 weight percent, such as at least 6 weight percent, for example at least 7 weight percent, such as at least 8 weight percent, for example at least 9 weight percent, such as at least 10 weight percent, for example at least 11 weight percent, such as at least 12 weight percent, for example at least 13 weight percent, such as at least 14 weight percent, for example at least 15 weight percent, such as at least 16 weight percent, for example at least 17 weight percent, such as at least 18 weight percent, for example at least 19 weight percent, such as at least 20 weight percent, and preferably less than 50 weight percent.

The preferred daily dosage of soybean fibers is from at least 1 g to about 100 g soybean fibers, for example from at least 2 to about 75 g soybean fibers, such as from at least 3 g to about 50 g, for example from at least 4 g to about 40 g, such as from at least 5 to about 30 g, such as from at least 10 g to about 20 g soybean fibers.

Preferred soy cotyledon fiber products manufactured by Protein Technologies International, Inc. are marketed under the brand name of FIBRIM®. Among the various soybean fibers produced under the FIBRIM® brand, FIBRIM® 1020 is particularly preferred because of a particularly pleasant mouth feel and dispersability for dry blended beverage applications. FIBRIM® 2000 is presently preferred for use in ready-made liquids.

Some compositions of isolated soy protein and soy cotyledon fiber are preferred in order to maximize the content of soy protein and isoflavones contained therein namely SUPRO® FXP-HO159, SUPRO® FXP-HO161, FIBRIM® 1450, FIBRIM® 2000 and FIBRIM® 1020 for dry blended beverage applications and SUPRO® FXP-HO159, SUPRO® FXP-HO161, FIBRIM® 1450, FIBRIM® 2000 and FIBRIM® 1020 for use in ready made liquids.

When a bread according to the present invention is for use in the prevention and/or treatment of type 2 diabetes, the metabolic syndrome and associated cardiovascular diseases, lecithinated fat reduced cacao is particularly preferred. Other preferred carbohydrates for use in a bread according to the present invention for use in the prevention and/or treatment of type 2 diabetes, the metabolic syndrome and associated cardiovascular diseases are polydextrose or saccharose, but these should be limited using other sweeteners like e.g. aspartame.

Vitamins and minerals may optionally be added to a bread according to the present invention in accordance with the limits laid down by health authorities. A bread according to the present invention may comprise all recommended vitamins and minerals. The vitamins will typically include A, B1, B2, B12, folic acid, niacin, panthotenic acid, biotin, C, D, E and K. The minerals will typically include iron, zinc, iodine, copper, manganese, chromium and selenium. Electrolytes, such as sodium, potassium and chlorides, trace elements and other conventional additives may also be added in recommended amounts.

A bread according to the present invention may be used for special dietary use, preferably for lowering serum levels of total cholesterol and/or LDL-cholesterol and/or triglycerides in subjects such as hyperlipidemic patients or normocholesterolemic patients suffering from a cardiovascular disease, and/or for lowering serum levels of glucose and/or insulin and/or total cholesterol and/or LDL-cholesterol and/or triglycerides and/or for increasing glucose tolerance and/or insulin sensitivity and/or for preventing, treating and/or alleviating impaired glucose tolerance and/or insulin secretory failure in diabetic subjects and/or for preventing, treating and/or alleviating an arteriosclerotic condition by reducing the influx of lipoproteins and/or cholesterol and/or triglycerides into the endocelium of the arterial wall of a diabetic subject suffering from a cardiovascular disease. For example, from one to three daily meals of ordinary food can be supplemented or replaced by a bread according to the present invention. Hereby, significant reductions in serum levels of cholesterol and/or LDL-cholesterol and/or triglycerides can be obtained, as well as an improvement of serum HDL/LDL-cholesterol ratio and/or an increase in serum HDL-cholesterol levels. The bread may provide from about 50 to about 250 kcal per serving.

The bread according to the present invention is effective in lowering levels of cholesterol in normocholesterolemic patients by at least 2%, for example at least 5%, such as at least 8%, for example at least 10%, such as at least 12%, for example at least 14%, such as at least 16%, for example at least 18%, such as at least 20%, for example at least 25%, such as at least 30%. The bread according to the present invention is effective in lowering levels of triglycerides in normocholesterolemic patients by at least 10%, such as at least 12%, for example at least 14%, such as at least 16%, for example at least 18%, such as at least 20%, for example at least 25%, such as at least 30%.

The bread according to the present invention is effective in lowering levels of cholesterol in mildly hypercholesterolemic patients by at least 3%, for example at least 5%, such as at least 8%, for example at least 10%, such as at least 12%, for example at least 15%, such as at least 20%, for example at least 25%, such as at least 30%, for example at least 35%, such as at least 40%, for example at least 45%. The bread according to the present invention is effective in lowering levels of triglycerides in mildly hypercholesterolemic patients by at least 15%, such as at least 20%, for

example at least 25%, such as at least 30%, for example at least 35%, such as at least 40%, for example at least 45%.

The bread according to the present invention is effective in lowering levels of cholesterol in severely hypercholesterolemic patients by at least 3%, for example at least 5%, such as at least 8%, for example at least 10%, such as at least 12%, for example at least 15%, such as at least 20%, for example at least 25%, such as at least 30%, for example at least 35%, such as at least 40%, for example at least 45%, such as at least 50%, for example at least 55%, such as at least 60%. The bread according to the present invention is effective in lowering levels of triglycerides in severely hypercholesterolemic patients by at least 20%, for example at least 25%, such as at least 30%, for example at least 35%, such as at least 40%, for example at least 45%, such as at least 50%, for example at least 55%, such as at least 60%.

In another embodiment the present invention provides the use of a bread according to the present invention in the treatment of cardiovascular diseases in the human or animal body in an amount effective in lowering serum levels of total cholesterol and/or LDL-cholesterol and/or triglycerides and/or homocystein and/or increasing the serum HDL/LDL-cholesterol ratio and/or serum HDL-cholesterol levels and/or reducing the influx of cholesterol and/or triglycerides into the arterial wall and/or reducing the amount of oxidized LDL-cholesterol present in the arterial wall and/or preventing, reducing or eliminating fatty streak formation and/or preventing, reducing or eliminating fibrous plaque formation and/or preventing, reducing or eliminating complicated lesion formation and/or reducing or eliminating the risk of a subject contracting angina pectoris and/or reducing or eliminating the risk of a subject contracting a myocardial infarction, and/or alleviating the clinical condition of patients contracting a myocardial infection. The cardiovascular disease is preferably a cardiovascular disease selected from the group comprising hypercholesterolemia, hypertriglyceridemia, other hyperlipidemias, arteriosclerosis, atherosclerosis, arteriolosclerosis, coronary heart disease, angina pectoris, thrombosis, myocardial infarction, and hypertension and more preferred selected from arteriosclerosis and atherosclerosis.

In another embodiment the present invention provides the use of a bread according to the present invention in the treatment of type 2 diabetes and/or the metabolic syndrome in an amount effective in lowering serum levels of total cholesterol and/or LDL-cholesterol and/or triglycerides and/or glucose and/or increasing serum levels of

HLDL-cholesterol and/or homocystein and/or reducing the influx of cholesterol and/or triglycerides into the arterial wall and/or reducing the amount of oxidized LDL-cholesterol present in the arterial wall and/or improving glucose tolerance and/or increasing insulin sensitivity and/or alleviating impaired glucose tolerance and/or improving insulin secretion and/or reducing or eliminating fatty streak formation and/or preventing, reducing or eliminating fibrous plaque formation and/or preventing, reducing or eliminating complicated lesion formation and/or preventing, reducing or eliminating the risk of a subject contracting angina pectoris and/or preventing, reducing or eliminating the risk of a subject contracting a myocardial infarction and/or preventing, treating, prophylactically treating, alleviating and/or eliminating hypertension and/or hyperglycemia and/or hyperinsulinemia and/or hypercholesterolemia and/or hypertriglyceridemia and/or arteriosclerosis and/or atherosclerosis and/or arteriolosclerosis in a diabetic subject.

In another embodiment the present invention provides the use of a bread according to the present invention in the treatment of a pulmonary disease in a human or animal body, preferably a disease selected from the group comprising inflammation of the airways, bronchoconstriction, bronchitis, asthma, and small airways diseases, in an amount effective in preventing, treating, prophylactically treating and/or alleviating inflammation of the airways and/or bronchoconstriction and/or bronchitis and/or small airways diseases and/or asthma and/or reducing and/or eliminating mucus hypersecretion and/or dyspnea in a subject suffering from asthma and/or increasing FEV1 of a subject as measured by forced expiratory volume in the first second of expiration.

The present invention also provides a method of preventing, treating, prophylactically treating and/or alleviating by therapy a cardiovascular disease in the human or animal body such as an arteriosclerotic condition of a human or animal body, said method comprising administration of a bread according to the present invention in an amount effective in lowering serum levels of total cholesterol and/or LDL-cholesterol and/or triglycerides and/or homocystein and/or increasing the serum HDL/LDL-cholesterol ratio and/or serum HDL-cholesterol levels and/or reducing the influx of cholesterol and/or triglycerides into the arterial wall and/or reducing the amount of oxidized LDL-cholesterol present in the arterial wall and/or preventing, reducing or eliminating fatty streak formation and/or preventing, reducing or eliminating fibrous plaque formation and/or preventing, reducing or eliminating complicated lesion formation and/or

reducing or eliminating the risk of a subject contracting angina pectoris and/or reducing or eliminating the risk of a subject contracting a myocardial infarction, and/or alleviating the clinical condition of patients contracting a myocardial infection. The cardiovascular disease is preferably a cardiovascular disease selected from the group comprising hypercholesterolemia, hypertriglyceridemia, other hyperlipidemias, arteriosclerosis, atherosclerosis, arteriolosclerosis, coronary heart disease, angina pectoris, thrombosis, myocardial infarction, and hypertension and more preferred selected from arteriosclerosis and atherosclerosis.

The present invention also provides a method of preventing and/or treating by therapy type 2 diabetes and/or the metabolic syndrome in a human or animal body, said method comprising administration to said human or animal body of a bread according to the present invention in an amount effective in lowering serum levels of total cholesterol and/or LDL-cholesterol and/or triglycerides and/or glucose and/or increasing serum levels of HLDL-cholesterol and/or homocystein and/or reducing the influx of cholesterol and/or triglycerides into the arterial wall and/or reducing the amount of oxidized LDL-cholesterol present in the arterial wall and/or improving glucose tolerance and/or increasing insulin sensitivity and/or alleviating impaired glucose tolerance and/or improving insulin secretion and/or reducing or eliminating fatty streak formation and/or preventing, reducing or eliminating fibrous plaque formation and/or preventing, reducing or eliminating complicated lesion formation and/or preventing, reducing or eliminating the risk of a subject contracting angina pectoris and/or preventing, reducing or eliminating the risk of a subject contracting a myocardial infarction and/or preventing, treating, prophylactically treating, alleviating and/or eliminating hypertension and/or hyperglycemia and/or hyperinsulinemia and/or hypercholesterolemia and/or hypertriglyceridemia and/or arteriosclerosis and/or atherosclerosis and/or arteriolosclerosis in a diabetic subject.

The present invention also provides a method of preventing, treating, prophylactically treating and/or alleviating by therapy a pulmonary disease in a human or animal body, preferably a disease selected from the group comprising inflammation of the airways, bronchoconstriction, bronchitis, asthma, and small airways diseases, said method comprising administration to said human or animal body of a bread according to the present invention in an amount effective in preventing, treating, prophylactically treating and/or alleviating inflammation of the airways and/or bronchoconstriction and/or bronchitis and/or asthma and/or small airways diseases and/or reducing and/or

eliminating mucus hypersecretion and/or dyspnea in a subject suffering from asthma and/or increasing FEV1 of a subject as measured by forced expiratory volume in the first second of expiration.

The period of treatment is preferably in the range of from 1 to 12 months or more, such as from 2 weeks to 9 months, for example from 3 weeks to 6 months, such as from 4 weeks to 4 months, such as from 6 weeks to 3 months. However, the period of treatment shall not be limited to these periods and may e.g. be longer than 12 months, such as e.g. a lifelong treatment in order to prevent cardiovascular diseases or in order to prevent and/or alleviate type 2 diabetes and/or a cardiovascular disease in connection therewith or in order to prevent pulmonary diseases.

In another embodiment, the present invention relates to the use of a bread according to the present invention as a partial or total diet for an overweight subject, an overweight subject suffering from an arteriosclerotic condition or an overweight subject suffering from a diabetic condition. Obesity is believed to be one of the major causes of diabetes including type 2 diabetes. Overweight subjects, including overweight diabetic subjects, often have increased serum cholesterol levels and increased triglyceride levels and are therefore more likely to develop cardiovascular diseases. However, the present invention is not limited to treating subjects with an increased risk of contracting a cardiovascular disease, i.e. subjects likely to have increased serum levels of cholesterol and/or triglycerides, or to treating obese diabetic subjects with an increased risk of contracting a cardiovascular disease, i.e. obese diabetic subjects likely to have increased serum levels of cholesterol and/or triglycerides. A bread according to the present invention also has substantial serum cholesterol, serum LDL-cholesterol and serum triglyceride lowering effects in subjects having a more normal lipid profile and in diabetic subjects that do not also suffer from overweight. The medical use of a bread to the present invention is not limited to overweight or obese subjects, including diabetic subjects, but may be used for normal weight subjects having increased serum levels of cholesterol and/or LDL-cholesterol and/or triglycerides or for subjects with a cardiovascular condition such as e.g. arteriosclerosis or a related condition who have normal serum levels of cholesterol and/or LDL-cholesterol and/or triglycerides. Such increased serum levels of cholesterol and/or LDL-cholesterol and/or triglycerides may be caused by intake of a diet rich in fats or it may be genetically related.

EXAMPLES

Example 1

White flour pan bread comprising Abacor blend

	Recipe		
	1	2	3
Ingredients (g)			
Water	4000	1800	1650 or 1800
Wheat flour	2280	1140	1140
Salt	72	36	36
Shortening	90	47,6	90
Instant dry yeast	47,6		47,6
Abacor Sw	1096		
Abacor N			514
Abacor Br		548	
SSL	18	9	9
Gluten	286	143	143
Veron CLX		0,25	
Method			
Point of Abacor at addition	Dry or dispersed in 2200ml water	Mixed with 700ml water No soaking	Dispersed in 750ml water
Resting time (min)	0	0	0 or 30
Results			
Weight (g)	730,8-734	754,7-759	761,6
Height (mm)	122,1-125,5	109,4-114	115,3
Dough temperature after mixing	25-27°C		
Picture	17	32	11

If Abacor is pre-hydrated with water it is either dispersed in the specified amount of water for about 30 seconds or it is mixed with water without soaking. The purpose with mixing Abacor and water alone is to obtain a homogenous mixture. All the other ingredients are then mixed with water using the commercial doughmixer Diosna SP 40 (Diosna, Dierks & Söhne Gmbh, Osnabruk, Germany) with a mixing time of 2 minutes at 100 rpm and 3,5 minutes at 200 rpm. The two mixtures are finally mixed together by use of the Diosna SP 40 doughmixer with a mixing time of 2 minutes at 100 rpm and 0,5 minutes at 200 rpm. The dough rests for 0 or 30 minutes. If Abacor is not pre-

hydrated with water all the ingredients are mixed simultaneously in the Diosna SP 40 and the remainder of the procedure is unchanged.

After mixing, the dough is divided into pieces of 850g or bigger using a single stage vacuum divider (Glimek AB, Glimåkra, Sweden). The weight of the dough pieces is controlled using a balance. The dough pieces are rounded using a conical rounder (Glimek AB). An intermediate proofing of about 5 minutes is applied (conveyor belt) before sheeting, curling and moulding by hand or by using a drum and roller sheeter (Glimek AB). The dough pieces are put into pans (height 97 mm, width 100 mm and length 180 mm) and proofed in a proofing cabinet (Lillnord A/S, Odder, Denmark) with the temperature of 37°C and the relative humidity RH 78%. Proofing time 35 minutes. After proofing, the loaves are baked for 35 minutes in a rotating hearth oven equipped with a fan (Bago-Line BEX 1,2, Faaborg, Denmark) at an initial temperature of 270°C which is reduced to 220°C immediately after placing the dough in the oven. Steam is added during the first 30 seconds of baking.

Loaves produced with Abacor Sw gives the highest height and the lowest weight. Using Abacor N and Br the water absorption must be reduced considerable.

Example 2

	Dough					
	1	2	3	4	5	6
Ingredients (g)						
Water	1840	1800	1840	1800	1740	1940
English flour	1140		1140		1140	1140
Norwegian flour		1140		1140		
Salt	36	36	36	36	36	36
Shortening						
Instant dry yeast from Gistbrocades			47,8	47,8		47,8
Instant dry yeast from Lesaffre	47,8	47,8			47,8	
Abacor Sw					274	548
Abacor N	274	274	274	274		
Abacor Br	274	274	274	274	274	
SSL	9	9	9	9	9	9

Gluten	143	143	143	143	143	143
Veron CLX	0,25	0,25	0,25	0,25	0,25	0,25
Method						
Point of Abacor at addition	Pre-mixed with 900ml water	Pre-mixed with 900ml water	Pre-mixed with 900ml water	Pre-mixed with 900ml water	Pre-mixed with 900ml water	Pre-mixed with 1100ml water
Resting time (min)	0	0	0	0	0	0
Results						
Weight (g)	760,4	723,5	738,4	734,9	752,3	728,0
Height (mm)	112,6	122,9	120,1	110,4	94,4	113,8
Picture	43	44	45	46	47	48

Abacor is pre-hydrated with water. All the other ingredients are mixed separately and finally mixed together with the mixture of Abacor and water. After mixing, the dough is divided into pieces using a single stage vacuum divider (Glimek AB, Glimåkra, Sweden). The weight of each dough piece is controlled using a balance. The dough pieces are rounded using a conical rounder (Glimek AB). An intermediate proofing of about 5 minutesis applied (conveyor belt) before sheeting, curling and moulding using a drum and roller sheeter (Glimek AB). The dough pieces are put into pans (height 97 mm, width 100 mm and length 180 mm) and proofed in a proofing cabinet (Lillnord A/S, Odder, Denmark) with the temperature at 37°C and the relative humidity RH 78%. Proofing time 35 minutes. After proofing, the loaves are baked for 35 minutes in a rotating hearth oven equipped with a fan (Bago-Line BEX 1,2, Faaborg. Denmark) at an initial temperature of 270°C which is reduced to 220°C immediately after placing the dough in the oven. Steam is added during the first 30 seconds of baking.

The English flour has higher water absorption than the Norwegian flour. In the recipes used there is compensated for the difference in water absorption by adding more water to the dough containing the English flour. The flours have different development times, measured with a Farinograph, indicating differences in flour quality. To obtain fully developed dough with a spiral mixer, (Diosna) the development time found by using the Farinogram is used. The proofing time used is appropriate for both types of instant dry yeast. No difference in fermentation rate, measured as bread height, is observed between the two different instant dry yeasts tested.

Dough comprising Abacor N and Abacor Br (Dough 1-4) are all sticky. The dough stick to the rollers thereby making it difficult to use the mechanical roller sheeter. If a mixture of Abacor Br and Abacor N is used the amount of water added must be reduced to avoid sticky dough. By using Abacor Sw alone (Dough 6) the dough is not sticky at all and it works very well in the mechanical roller sheeter. However the breadcrumb contains large blisters. Using Abacor Sw instead of Abacor N in the Abacor mixture and reducing the amount of water (Dough 5) dough stickiness disappears and it is no longer a problem to use the mechanical roller sheeter. The amount of water added is to low and the dough becomes stiff. However, a mixture of Abacor Br and Abacor Sw is probably the best, in the experiments presented in this example.

Example 3

Whole meal flour bread

	Dough								
	A	B	C	D	E	F	G	H	I
Ingredients (g)									
Water	2100	1750	1750	1750	1750	1550 or 1750	1750 or 3700	1750 or 3500	1700 or 3400
Wheat flour	532	532	532	532	532	532	532	532	532
Salt	38	38	38	38	38	38	38	38	38
Shortening									
Instant dry yeast	50	50	50	50	50	50	50	100	0
Abacor Sw	569						284,5		
Abacor N		569				284,5			
Abacor Br			569	569	569	284,5	284,5	569	569
SSL	9,4	9,4	9,4	9,4	9,4	9,4	9,4	9,4	9,4
Gluten	150	150	150	150	150	150	150	150	150
Veron CLX	0,285	0,285	0,285	0,285	0,285	0,285	0,285	0,285	0,285
Wheat whole meal flour	550	550	550	550	550	550	550	550	550
Oat flakes	94,1	94,1	94,1	94,1	94,1	94,1	94,1	94,1	94,1
Method									
Point of Abacor at addition	Mixed with 1100ml water	Mixed with 850ml water	Mixed with 850ml water	Dry. No soaking	Mixed with 850ml water	Mixed with 800ml water	Mixed with 1800ml (??) water	Mixed with 1600ml (??) water	Mixed with 800ml (??) water

Point of whole meal flour at addition	Dry	Dry	Dry	Dry	Soaked	Dry	Dry	Dry	Dry
Resting time (min)	0	0	0	0	0	0	0	0	0
Results									
Weight (g)	736,5-742,7	755,3-755,7	735,5-735,9	740,0-746,4	730,7-735,8	736,6-740,4	738,5-741	735,9-736	736,4-738,8
Height (mm)	98,6-99,6	87,8-88,4	94,6-96,0	90,0-90,5	90,5-94,3	99,0-104,5	103,8-104,5	94,9-96,9	89,3-90,9
Picture	30	31	34	33	35	36	37	38	40

If Abacor is pre-hydrated with water the mixing takes place without soaking, the mixing time is about 30 seconds. The purpose of pre-hydrating is to obtain a homogenous mixture. The remainder ingredients are mixed with water using a commercial dough mixer Diosna SP 40 (Diosna, Dierks & Söhne Gmbh, Osnabruk, Germany), mixing time of 2 minutes at 100 rpm and 3,5 minutes at 200 rpm. Finally, the two mixtures are mixed using a Diosna SP 40 dough mixer, mixing time 2 minutes at 100 rpm and 0,5 at 200 rpm. After mixing the dough temperature is 25°C. No resting time is used. If Abacor is not pre-hydrated, all the ingredients are mixed in the Diosna SP 40 and the remainder of the procedure is unchanged.

After mixing, the dough is divided into 850g dough pieces using a single stage vacuum divider (Glimek AB, Glimåkra, Sweden). The weight of each dough piece is controlled using a balance. The dough pieces are rounded using a conical rounder (Glimek AB). An intermediate proofing of about 5 minutes is applied (conveyor belt) before sheeting, curling and moulding by hand or by using a drum and roller sheeter (Glimek AB). The dough pieces are put into pans (height 97 mm, width 100 mm and length 180 mm) and proofed in a proofing cabinet (Lillnord A/S, Odder, Denmark) with the temperature at 37°C and the relative humidity RH 78%. Proofing time 35 minutes. After proofing, the loaves are baked for 35 minutes in a rotating hearth oven equipped with a fan (Bago-Line BEX 1,2, Faaborg. Denmark) at an initial temperature of 270°C which is reduced to 220°C immediately after placing the dough in the oven. Steam is added the first 30 seconds of baking.

The breadcrumb obtained using Abacor Sw has a nice pore structure and compared to Abacor N the bread weight is reduced and the bread height is increased when using Abacor Sw.

If Abacor Br is mixed with Abacor N or Abacor Sw the height of the loaves increase, picture 36 and 37 respectively. The best breadcrumb is obtained with a mixture of 50% w/w Abacor N and 50% w/w Abacor Br. The crust does not contain so many pores as with Abacor Br alone and when tasting the bread slice for extensibility it is much better than when the bread comprises a mixture of 50% w/w Abacor Sw and 50% w/w Abacor Br. The breadcrumb obtained with this mixture has a weakness just below the crust. The bread volume obtained with Abacor N alone is lower and the weight higher compared to the volume obtained when using mixture of 50% w/w Abacor N and 50% w/w Abacor Br.

The bread volume and height increases and bread weight reduces when whole meal flour and oat flakes are soaked for 20 minutes before mixing. Use of oat flakes has a positive effect on breadcrumb and crust, thus the breadcrumb becomes brighter and the breads crust becomes more shiny and even.

EXAMPLE 4 - Pilot scale preparation of new ISP's

Processing Conditions

The process and process conditions of this example were based on Procedure 3 from EXAMPLE 3. One important difference was that no second extraction at pH 5.4 was carried out. The preparation of the ISP was carried out in a number of batches which were combined to give the products as the Final Mix (FM).

Procedure

Table 1: Separation Scheme for Isolate

Step	Action	Fractions	Time (min)
1	Soy flour (40 kg) was dispersed in water (300l) at 20 to 21°C in Tank 1 with a portion of anti-foam FDP (75 ml).		~ 10

2	The mixture was adjusted to pH 8.0 with 2 M KOH (~3.6 l)		~ 5.0
3	Extraction with continuous stirring (30 min.) was followed by holding without stirring (30 min.)		60
4	The slurry was centrifuge to remove solids with feed rate of 726 l/h and a pressure of 4.25 kg/cm^2 into Tank 2. The partially cleared slurry was re-centrifuged and returned to Tank1. The fibre-rich solids collected by the centrifuge were discarded.	Fibre + high Mwt. proteins	~ 90
5	Small ice pieces (48kg) were added to bring temperature ~ 7.0° C		~ 5.0
6	The pH of the solution was reduced to 5.4 with 5.5 M HCl (~1.7 l)		~ 5.0
7	Precipitation was allowed to occur without stirring		90
8	Isolate was separated by centrifuging at 726 l/h and a pressure of 4.25 kg/cm^2 from Tank1 to Tank 2 and then back to Tank 1. The precipitate recovered from the centrifuge. Antifoam FDP (75 ml) was added during the separation.	ISP, fraction 1	~ 90
9	Small ice pieces (12 kg) were added to the solution to bring temperature ~ 10° C		~ 3.0
10	The pH was reduced to 3.5 with 5.5 M HCl (approximately 3.7l)		~ 5.0
11	Precipitation was allowed to occur without stirring		60
12	The isolate was separated by centrifugation at a feed a rate of 726 l/h and a pressure of 4.25 kg/cm^2 in a single pass from Tank 1 to Tank 2. The insoluble isolate was collected and the liquor of solubles discarded.	ISP, fraction 2	~ 40
13	Both isolates were combined in a 25l-vessel and mixed with a Silverson. Samples were collected for microbiology, protein and moisture.		~ 10

14	The pH was raised to between 6.0 to 6.5 with a concentrated solution of KOH (415g in approx. 500 ml water).		~ 10
15	Portions (6.0 kg) of the slurry were deposited in freezing trays (1000mm x 495mm) lined with plastic sheets and frozen at -21°C in walk-in freezer.		~ 60

RESULTS

The results from the analysis if the FM for moisture, fibre, ash, and protein content.

Table 2. Analysis of freeze-dried isolate

	Moisture (%)	Fibre	Fibre dry basis	Ash	Ash (dry basis)	Protein (as is)	Protein (dry basis)	Fibre Ash+Prot
Final mix FM	**5.3**	**4.30**	**4.5**	**6.64**	**7.0**	**77.0**	**81.3**	**92.8**

Physical analysis of free-dried isolate

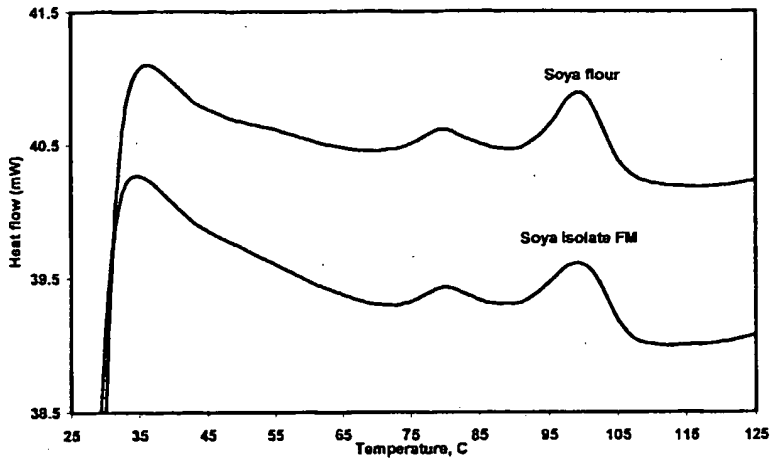
Differential thermal analysis

Table 3: DSC data for flour, isolate and some commercial isolates

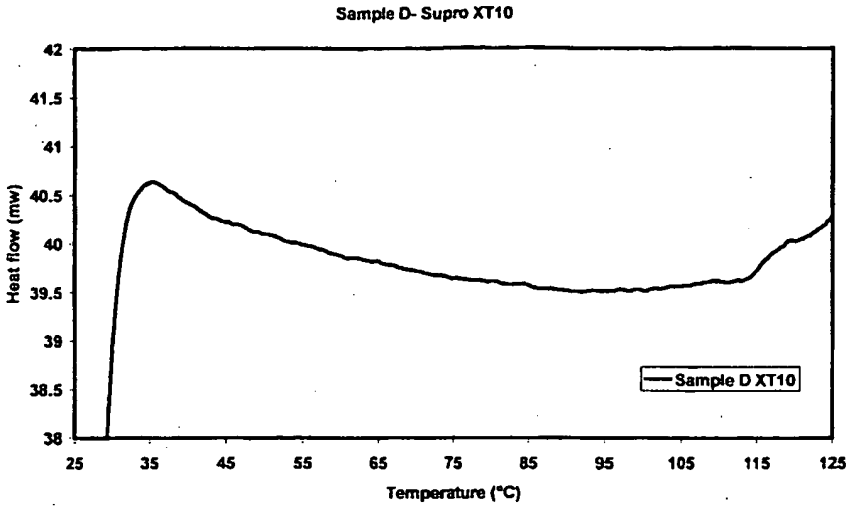
	Weight used in DSC pan (mg)	Enthalpy Peak 1 (J/g dry protein)	Enthalpy Peak 2 (J/g dry protein)
Commerciall soy flour	46.3	1.48	5.64
Soya isolate final mix	32.5	1.10	5.41
A - FXP H0161	52.8	0	0
D - XT 10	34.6	0	0

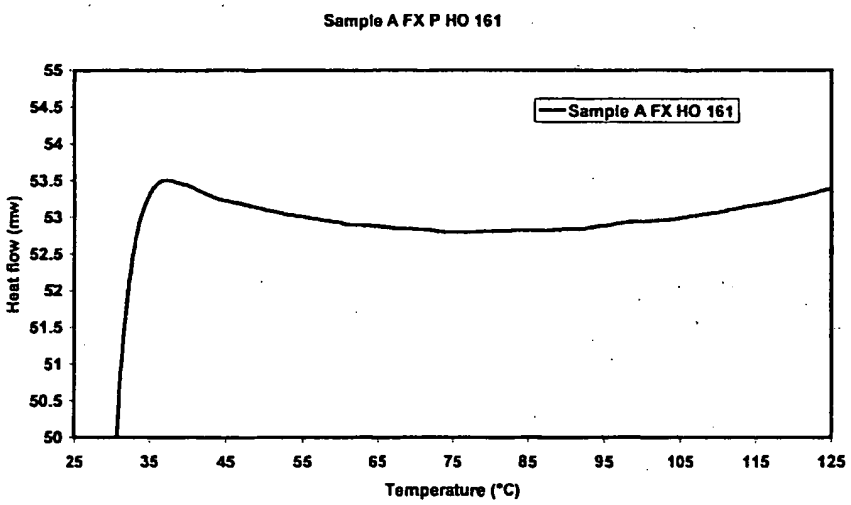
The two graphs for soy flour and soy isolate FM are plotted on the same graph with the same scale for the peaks. The difference in baseline due to sample mass has been reduced by shifting the flour graph downwards by 7.0 units.

Figure 1: Comparison of DSC graphs of soy flour and isolate FM on same protein basis, showing the 7S (75-85°C) and 11S (95-105°C) enthalpy peaks.



As shown in the graphs below, two commercial available qualities of ISP from Du Pont Protein Technologies, the curves are without peaks, indicating that there is not any un-denatured globular protein structure left in the products.





The PAGE data showed strong bands for the 7 and 11 S proteins that were more pronounced than for the flour, supporting the DSC finding.

Table 4: Values of the peak areas shown in graph of densitometry in the soy flour and the FM isolate at equivalent protein levels for loading the PAGE.

Mol. weight in Da	79.5	71.7	64.6	43.1	29.3	14	9.6
Protein type	7S	7S	7S	7S	11S	11S	PEPTIDES

Soy flour OD value	49	81	125	118	160	176	23
Isolate FM OD value	50	105	135	180	210	210	20

OD is the optical density reading of the scanning device used to "read" the gels.

Solubility of isolated precipitated at 5.4 and 3.5

Table 5: Results of analysis of commercial isolates for %protein solubility

Samples		Total protein, %	Soluble protein, %		% soluble
A	FX H0 161	84.5	46.5		55
B	Supro 760	84.5	23		27.2
C	661	87.5	19		21.7
D	XT10	82	34		41.5
E	FX H0 159	77.5	40		51.6
F	219	83	43.5		52.4
G	219D	82.56	44		53.3
H	LH (Bunge)	85	35.5		41.8
I	NB (Bunge)	86.5	24		27.8
J	Profam 940 (ADM)	84	28		33.3
K	Fibrim 1020	8.5	3		35.3
L	Supro ST	79	16		20.3
M	Supro 770LN	84.5	25		29.6
N	Supro XT34	80	22		27.5
Isolates from Example 4 batches 18/20		**82.1** **80.1**	**78.8** **77.7**		**96.0** **97.1**

The solubility of the isolates prepared according to this example were approximately 96-97% after freeze-drying showing no denaturation had occurred

Isoflavon analysis

Table 6: Selected values for comparison on total isoflavon level

Identification	Genistein	Daidzein	Glycitein	Total as Aglycon mcg/g	Total as Glycoside mg/g
Commercial soy flour used for preparing ISP	770	654	13	1437	2.44
ISP	667	641	94	1402	2,38

From the above data it is clear that the process according to the present invention allows for the preparation of soy protein products retaining almost all the isoflavones present in the starting material. This is in line with the undenatured state of the soy protein as the isoflavones are associated with the interior of the proteins and would thus remain associated with the proteins when the globular structure is intact.

PAGE densitograms:

The isolate was run at 3 concentrations of material added to the gel. In general terms the O.D. values are linear from 0.01 to 0.8 and it is unwise to use higher values. The most diluted sample gave the best separation of the peaks and the densitogram from this was consequently used for further analysis. This was for the isolate obtained by mixing 0.025ml of the extract with 0.375ml of Laemmli buffer.

The same was true for the densitograms for the soy flour. The lowest concentration of material added to the gel gave a better separation.

Table 7: 7 and 11S protein content in Flour and ISP

Product	7S	11S	Other, soy proteins
Flour, %	28	29	43
ISP, %	34	39	27

Table 8: Composition of the basic components in 7S and 11S of the ISP

	7 S			11S	
Components	α'	α	β	A	B
Amounts % of total prot.	9	12	13	19	20

The amounts of 7 and 11S proteins are considerably increased in concentration during the processing. This is because the highest molecular weight proteins, the 15 S fraction will be separated out together with the insoluble fibres and much of the 2S fraction is more water-soluble and will be a part of the water solution and removed.

The SDS-PAGE data indicated that the second washing and precipitation of ISP at pH 5.4 would have contributed to higher yield of ISP, but also to an increased amount of 7S as this ISP fraction showed a different relation between 7 and 11S than from the first precipitation. The result would most probably have been a result of more equal quantities of 7 and 11S than shown in the Table 7 above.

Material and Equipment.

1. Two deliveries (1000 kg) of soy flour were purchased from Cargill as lots 1 and 2. Lot 1 was processed as batches Nos.14 to 31 and lot 2 as batches Nos.32 to 40.
2. Reagent grade Hydrochloric acid (specific gravity 1.18) and
3. Potassium hydroxide (56.1g/mol.) was purchased from BDH and tap water was used to make the slurry.
4. A foam-depressing reagent, antifoam FDP, was obtained from Basildon Chemical Company Ltd., Kimber Road Abingdon Oxon., OX14 1RZ.

The table below shows the main data for the two batches of Soy Flour used as raw material for the process and the pilot scale production:

Table 9. Soy flour -starting material

Detail		
Name	De-fatted soy Provabis 200/80	De-fatted soy Provabis 200/80
Batch number	820423	8198859
Production	18/04/02	18/04/02
Protein, %	> 52	> 52
Fibre, %	3.0-3.5	3.0-3.5
Oil, %	0.7-1.2	0.7-1.2
Moisture, %	< 10	< 10
Total plate count, ln	max. 5.2	max. 5.2
Enterobacteriacae, ln	max. 4.0	max. 4.0
Salmonella	nil	nil
Granulation %	< 6	< 6
Used in batches	1-13	14-40

Equipment

1. Centrifugal separators from Westphalia Separators, Model SA 7-06-476 with self-cleaning bowl and a set of 69 separating cones, and
2. A paddle mixer was used to make the flour/water slurry and for mixing during pH adjustment.
3. A Silverson mixer (without shearing element) model D, Silverson Machine Ltd, Waterside, Chesham, Bucks, HP5 1PQ was used to mix the isolates during pH adjustment.
4. APV 454 litres capacity steam jacketed kettles were used as holding tanks.
5. A NORD, model SK 20R150U90L/433, CIP lobe pump was used to feed slurry to the separator.
6. An Edwards freeze Dryer Modulyo was used for small-scale drying of isolates.
7. Commercial Freeze Drying was performed with N° Three and N° Seven freeze dryers.

Freeze Drying:

Comment:

Freeze-drying was chosen as a suitable method in this case. To avoid microbiological growth in all produced ISP in the established pilot process was frozen on trays immediately after production. Instead of having to defrost all material again with increased microbiological risk, we decided to use freeze drying in this particular case. Freeze-drying is also a rather gentle drying method.

Generally all kind of drying methods can be used, but the drying conditions must in all cases be chosen carefully to avoid further denaturation. The standard spray drying method is preferred for full production scale operations. But in such cases it is an advantage not to dry to low water level as this might influence the dispersion and flavour properties of the final product.

Laboratory scale to obtain analytical values

Samples were frozen at -21°C. Edwards freeze Dryer Modulyo was used for drying the isolate at lab scale. Samples were kept at -55°C and a vacuum pressure of 10^{-1} mbar (0.1mm Hg) was used. It took 4 to 5 days to dry even very small samples.

Commercial Freeze Drying:

The process was undertaken in two dryers, N° Three and N° Seven designed and built by Commercial Freeze Drying Ltd. Both dryers were CFD. N° Three has 28 trays of approximately 1000mm x 495mm and N° Seven holds 102 trays of the same size.

The frozen product was received by Commercial Freeze Dry Ltd. was held in cold storage at -20°C. Clean trays were then lined and the product weighed onto them at 5 kg/tray. The trayed product was then held overnight at -20°C to stabilise and the freeze dryer was cleaned with Commercial Freeze Dry Ltd. normal cleaning procedures and cooled to -20°C ready for loading. After loading a vacuum was created in the freeze dryer and it was run at a vacuum pressure < 2 mbar. Since the product was heat sensitive, a heat profile was run which prevented the product from reaching a temperature of greater than +30°C. This considerably extended the drying time.

First batch of 140 kg of wet product was dried In N° Three dryer on 5th September through 7th September. Dried product was then ground with an Apex comminuting mill model 114 type S2 at a fast hammer rotation speed and a screen aperture of 0.125".

After examination of the powder sample, it was too coarse and to reduce the particle size further, the finished product screen aperture was reduced to 0.107"for subsequent milling of the product. The product was then dispatched to CCFRA.

The remaining wet product was dried in five lots of 140 kg each in N° Three dryer and two lots of 510 kg each in N°Seven dryer. The dried product was then ground using 0.107" screen and dispatched to CCFRA in sealed in plastic sacks for further processing. Grinding screens were restricted by concerns for the temperature sensitivity of the product. A fine screen necessary to obtain particles < 150 um caused a build up of heat in the product, so a larger screen was used.

EXAMPLE 5

Comparison of the clinical effect of ingesting undenatured soy protein according to the present invention or a commercial ISP with a high isoflavone content.

Two Isolated soy protein products A (SuproSoy) and B (Undenatured ISP) were studied with a placebo C (casein) in a randomized placebo-controlled trial according to GCP with respect to their lipid lowering effects.

Study details: The patients took for 8 weeks daily 25g of the 3 products in 2 dosages in the morning and in the evening. The products were dissolved in water. Intermediate visits occurred after 2 and 4 weeks.

Patients: 120 patients of both sexes (73 women and 47 men in the age of 32 to 70) were included if they fulfilled the inclusion criteria of 200-300mg/dl or 5.2-7.8 mmol/l total cholesterol.

Statistics: For the primary (total cholesterol) and secondary parameters (LDL- and HDLcholesterol) the mean differences between the first and fourth visit were tested using the group comparison analysis of variance.

Dropouts: During the study the following dropouts occurred: Group A: 8 cases, group

B: 10 cases, and group C: 11 cases; from those only partial results are available. Therefore the analysis was done for the per protocol group; in addition a intention-to-treat analysis was performed.

Side effects: No severe side effects occurred.

Description of patients: 120 patients included; 91 finished per protocol.

Results: Of the protocol analysis:
The mean age was 55.1 years
Weight: the weight increase during the study was 0.2-0.6 kg

1) total cholesterol:

Difference visit 4 vs visit 1:

A: - 12.8 mg/dl -5.0%
B: - 24.3 mg/dl -9.4%
C: + 1.1 mg/dl +0.4%

Percentage changes Active vs Placebo:

A-C: -5.4%
B-C: -9.8%

Significances:

A:B O.017
A:C 0.013
B:C 0.001

2) LDL cholesterol

Difference visit 4 vs visit 1:

A: -12.3 mg/dl - 7.5%

B: -19.4 mg/dl -11.8%

C: - 5.8 mg/dl - 3.6%

Percentages changes Active vs Placebo:

A-C: 3.9 %

B-C: 8.2 %

Significances:

A:B 0.081

A:C 0.159

B:C 0.006

3) HDL cholesterol

No significant changes occurred

Summary:

The new undenaturated ISP formulation has proven to be significantly more effective to reduce total cholesterol than SuproSoy ISP. LDL cholesterol was significantly lowered with the new undenaturated ISP, but not for SuproSoy, in 91 patients with hypercholesterolemia in 8 weeks in a randomized placebo-controlled trial.

The percentage improvement of total cholesterol of the new undenaturated ISP was 9.8% compared to SuproSoy ISP with 5.4%; an improvement of over 80%. The improvement of LDL cholesterol was 8.2% for the new undenaturated ISP compared to 3.9% for SuproSoy, an improvement of over 100%.

CLAIMS

1. Bread comprising soy protein in an amount of at least 8% by weight of the bread.

2. Bread according to claim 1 comprising phytoestrogens in an amount of at least about 0,10% by weight of the soy protein source.

3. Bread according to claim 2 wherein at least one of the phytoestrogen compounds is selected among isoflavones.

4. Bread comprising soy protein in an amount of at least 4% by weight of the bread and soy dietary fibre in an amount of at least 0,9% by weight of the bread and soy phospholipids in the amount of at least 0,07% by weight of the bread.

5. Bread according to claim 4 comprising phytoestrogens in an amount of at least about 0,10% by weight of the soy protein source.

6. Bread according to claim 5 wherein at least one of the phytoestrogen compounds is selected among isoflavones.

7. Bread according to any of claims 4 to 6 comprising a phosphatidyl choline compound in an amount of at least 10% by weight of the soy phospholipid source of the bread.

8. Bread according to any of claims 4 to 7 wherein the soy phospholipid is lecithin.

9. Bread according to any of claims 4 to 7 wherein the soy dietary fibre is cotyledon.

10. Bread according to any of claims 1 to 9 comprising an emulsifier such as Sodium Stearoyl Lactylate (SSL) in an amount of at least 9% by weight of the soy fibre content of the bread.

11. Bread according to any of claims 1 to 10 comprising exogenous added gluten in an amount of more than 101% by weight of the soy fibre content of the bread.

12. Bread according to any of claims 1 to 11 comprising an amylolytic enzyme preparation in an amount of at least 0,1% by weight of the soy fibre content of the bread.

13. Bread according to claim 12 wherein the amylolytic enzyme preparation has additional transglutaminase and hemicellulase activity.

14. Bread according to any of claims 4 to 13 wherein the soy protein, soy dietary fibre and soy phospholipids are incorporated as a blend.

15. Bread according to claim 14 wherein the blend is pre-hydrated with at least 7g liquid per gram of soy fibre in the bread.

16. Bread according to claim 14 wherein the blend is dispersed in liquid before being incorporated to the dough.

17. Bread according to claim 16 wherein the liquid is selected from the group consisting of water, milk and juice.

18. Bread according to claim 17 wherein the liquid is water.

19. A method for manufacturing bread comprising soy protein in an amount appropriate to achieve a content of at least 8,4% by weight of the bread wherein said method comprises a step of pre-hydrating the soy protein prior to incorporating it to the dough.

20. A method according to claim 19 wherein said soy protein is pre-hydrated by mixing with liquid.

21. A method according to claim 20 wherein said soy protein is pre-hydrated by mixing with liquid without soaking.

22. A method according to claim 19 wherein said soy protein is pre-hydrated by dispersing it in liquid for about 30 seconds.

23. A method according to any of claims 19 to 22 wherein the liquid is selected from the group consisting of water, milk and juice.

24. A method according to claim 23 wherein the liquid is water.

25. A method for manufacturing bread with a blend comprising

- soy protein in an amount appropriate to achieve a content of at least 4,9% by weight of the bread
- soy dietary fibre in an amount appropriate to achieve a content of at least 0,9% by weight of the bread
- soy phospholipids in an amount appropriate to achieve a content of at least 0,07% by weight of the bread

wherein said method comprises the step of pre-hydrating the blend prior to incorporating it to the dough.

26. A method according to claim 25 wherein said blend of soy protein, soy dietary fibre and soy phospholipids is pre-hydrated by mixing with liquid.

27. A method according to claim 26 wherein said blend of soy protein, soy dietary fibre and soy phospholipids is pre-hydrated by mixing with liquid without soaking.

28. A method according to claim 25 wherein said blend of soy protein, soy dietary fibre and soy phospholipids is pre-hydrated by dispersing it in liquid for about 30 seconds.

29. A method according to any of claims 25 to 28 wherein the liquid is selected from the group consisting of water, milk and juice.

30. A method according to claim 29 wherein the liquid is water.

31. A method according to any of claims 19-30 wherein said blend is added to the mixer immediately prior to the mixing procedure.

32. A method according to any of claims 19-30 wherein said soy blend is added to the mixer during the mixing procedure.

33. A method according to any of claims 31 and 32 wherein the moisture level of the dough is adjusted in order to make the dough machineable.

34. A method according to claim 33 wherein the moisture level of the dough is adjusted to at least 43%.

35. A method according to any of claims 33 and 34 wherein the bread is manufactured by the sponge and dough method.

36. A method according to claim 35 wherein the sponge comprises wheat flour, yeast and water.

37. A method according to claim 36 wherein the sponge is allowed to stand before the dough is mixed.

38. A method according to claim 37 wherein the sponge is allowed to stand for at least 4 hours before the dough is mixed.

39. A method according to any of claims 33 and 34 wherein the dough is made by use of spiral mixing.

40. A method according to claim 39 wherein the mixing procedure comprises mixing at various velocities.

41. A method according to claim 40 wherein the mixing velocities are chosen from a group comprising slow mixing at 100 rpm. and fast mixing at 200 rpm.

42. A method according to any of claims 33 and 34 wherein the dough is made by use of CBP mixing.

43. A method according to claim 42 wherein the mixing is commenced for the time required for 11 watts per kilo to be achieved.

44. A method according to any of claims 33 and 34 wherein the dough is made by use of atsmospheric or atmospheric/vacuum mixing.

45. A method according to claim 44 wherein the mixing is commenced for 2-3 minutes till approx. 10,8-11 watt hours per kilo is achieved. When using atmospheric/vaccum mixing a 15" vaccum is pulled at 48 watt hours.

46. A method according to any of claims 33 and 34 wherein an amymolytic enzyme preparation is added together with the remainder dry ingredients when mixing the dough.

47. A method according to any of claims 33 and 34 wherein an amymolytic enzyme preparation is blended with the flour prior to mixing the dough.

48. A method according to claim 47 wherein the amymolytic enzyme preparation is blended with the flour for 30 minutes before mixing the dough.

49. Bread manufactured according by a method according to any of claims 19-48.

50. Use of a bread according to any of claims 1 to 18 or 49 for lowering serum levels of glucose and/or total cholesterol and/or LDL-cholesterol and/or triglycerides and/or homocystein and/or for increasing serum levels of HDL-cholesterol and/or the serum HDL/LDL-cholesterol ratio in a subject.

51. Bread according to any of claims 1-18 wherein the soy protein used in the manufacture has a content of intact 7S subunits (α + α' + β) and 11S subunits (A + B) which constitute more than 61.2 % of the total protein content.

52. Bread according to any of claims 1-18 wherein the soy protein used in the manufacture has a content of intact 7S subunits (α+ α' + β) which constitute more than 28.2% of the total protein content.

53. Bread according to any of claims 1-18 wherein the soy protein used in the manufacture has a content of intact 7S subunit α subunit which constitute more than 8.6 % of the total protein content.

54. Bread according to any of claims 1-18 wherein the soy protein used in the manufacture has a content of intact 7S subunit α' subunit which constitute more than 7.4 % of the total protein content

55. Bread according to any of claims 1-18 wherein the soy protein used in the manufacture has an isoflavone content of more than 0.16 % (w/w) of the total soy protein, and a content of intact 7S subunits (α + α' + β) and 11S subunits (A + B) which constitute more than 10 % of the total protein content.

56. Bread according to any of claims 1-18 wherein the soy protein used in the manufacture has an isoflavone content of more than 0.16 % (w/w) of the total soy protein, and a content of intact 7S subunits (α+ α' + β) which constitute more than 5 % of the total protein content.

57. Bread according to any of claims 1-18 wherein the soy protein used in the manufacture has an isoflavone content of more than 0.16 % (w/w) of the total soy protein, and a content of intact 7S subunit α which constitute more than 2 % of the total protein content.

58. Bread according to any of claims 1-18 wherein the soy protein used in the manufacture has an isoflavone content of more than 0.16 % (w/w) of the total soy protein, and a content of intact 7S subunit α' which constitute more than 2 % of the total protein content.

59. Bread according to any of claims 51-58 in which the content of active trypsin inhibitor has been reduced to less than 20% of the amount present in the original soy bean material.

60. Bread according to any of claims 51-58 in which the arginine:lysine ratio in the soy protein used is at least 1.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IB 03/00669

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A21D2/26 A21D2/36 A21D8/04 A23L1/305

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A23L A21D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
WPI Data, EPO-Internal, PAJ, FSTA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2001/019734 A1 (GILBERTSON DENNIS B ET AL) 6 September 2001 (2001-09-06)	1-11,49
A	page 3, column 2, paragraph 3; examples 1,2 page 4, column 2, paragraph 5 -page 5, column 1, paragraph 3; claims 1-30	19-48
Y	WO 00 30665 A (HOEIE LARS HENRIK ;NUTRI PHARMA AS (NO)) 2 June 2000 (2000-06-02) cited in the application page 39, line 4-18; claims 1-66	2-18,50
X	US 5 698 256 A (STILLING BIRGITTE) 16 December 1997 (1997-12-16) cited in the application	1-49
Y	column 4, line 52-56; claims 1-14; examples 1-3	2-18,50

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 June 2003	07/07/2003

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Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	POTTER S M ET AL: "DEPRESSION OF PLASMA CHOLESTEROL IN MEN BY CONSUMPTION OF BAKED PRODUCTS CONTAINING SOY PROTEIN 1-3" AMERICAN JOURNAL OF CLINICAL NUTRITION, BETHESDA,MD, US, vol. 58, no. 4, October 1993 (1993-10), pages 501-506, XP000878776 ISSN: 0002-9165	50
A	the whole document	1-49
X	GB 901 110 A (SHORT MILLING CO J) 11 July 1962 (1962-07-11) cited in the application	1-18,49
A	page 2, column 2, line 78-86; example 1	19-48
X	US 3 930 055 A (ENGELMAN ELAINE GREEN ET AL) 30 December 1975 (1975-12-30) cited in the application the whole document	1,49
X	GB 1 522 439 A (SPECIAL FOOD CORP) 23 August 1978 (1978-08-23) page 2, column 2, line 31-36; example 1	1,4,49
X	SOZA E DE S ET AL: "Eiweiss- und Ballaststoffanreicherung bei argentinischem Weizenmehl." GETREIDE, MEHL UND BROT 1981, vol. 35, no. 6, pages 150-153, XP008019007 the whole document	1,4,49
A	EP 0 528 766 A (STEINER & WEHRLI AG) 24 February 1993 (1993-02-24) page 3, line 1-45; claims 1-6	1-60
X	ZIMMERMANN R ET AL: "Uber funktionelle Eigenschaften von Sonnenblumenproteinen in Weizenteig. III. Vergleichende Untersuchungen zum Konzentrationseinfluss von Sonnenblumen- und Sojaproteinisolaten auf Wasseraufnahme und rheologische Eigenschaften." NAHRUNG 1984 SEKTION NAHRUNGSGÜTERWIRTSCHAFT & LEBENSMITTELTECH., HUMBOLDT UNIV., BERLIN, vol. 28, no. 9, pages 967-974, XP008019005 the whole document	1,4,49

-/--

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ZIMMERMANN R ET AL: "Uber funktionelle Eigenschaften von Sonnenblumenproteinen in Weizenteig. IV. Vergleichende Untersuchungen zur Wirkung einer Vorquellung von Sonnenblumen- und Soja-Proteinisolaten bei verschiedenen pH-Werten." NAHRUNG 1984 SEKTION NAHRUNGSGÜTERWIRTSCHAFT & LEBENSMITTELTECH., HUMBOLDT UNIV., BERLIN, vol. 28, no. 9, pages 975-981, XP008019004 the whole document	1,4, 19-49
Y	US 2001/055642 A1 (COHEN DEBORAH W) 27 December 2001 (2001-12-27)	19-48
A	page 1-2; claims 1-29	1-18,49
Y	EP 1 129 626 A (PROTEIN TECH INT) 5 September 2001 (2001-09-05) page 18, line 55 -page 19, line 1; claims 1-34	19-48
A	WO 00 19839 A (MONSANTO CO) 13 April 2000 (2000-04-13) the whole document	51-60
A	EP 1 174 516 A (FUJI OIL CO LTD) 23 January 2002 (2002-01-23) the whole document	51-60

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB 03/00669

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claim 50 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/IB 03/00669

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
US 2001019734	A1	06-09-2001	WO	02051250 A1	04-07-2002
			CA	2305900 A1	16-10-2000
WO 0030665	A	02-06-2000	AU	1404700 A	13-06-2000
			BR	9915687 A	04-12-2001
			CA	2352585 A1	02-06-2000
			EP	1133308 A1	19-09-2001
			WO	0030665 A1	02-06-2000
			JP	2002530347 T	17-09-2002
			US	2003113390 A1	19-06-2003
US 5698256	A	16-12-1997	DK	88292 A	07-01-1994
			AU	4416893 A	31-01-1994
			DE	69304543 D1	10-10-1996
			WO	9400995 A1	20-01-1994
			EP	0656748 A1	14-06-1995
			FI	950068 A	05-01-1995
			NO	950039 A	05-01-1995
GB 901110	A	11-07-1962	NONE		
US 3930055	A	30-12-1975	NONE		
GB 1522439	A	23-08-1978	DE	2606546 A1	24-03-1977
			AT	347371 B	27-12-1978
			AT	526676 A	15-05-1978
			BE	851557 A1	16-06-1977
			CA	1113303 A1	01-12-1981
			CH	602017 A5	14-07-1978
			EG	13899 A	31-12-1982
			FR	2341273 A1	16-09-1977
			IT	1115941 B	10-02-1986
			JP	52139738 A	21-11-1977
			JP	55034654 B	08-09-1980
			LU	76805 A1	06-07-1977
			NL	7701735 A	23-08-1977
			SE	7701854 A	20-08-1977
EP 0528766	A	24-02-1993	CH	681192 A5	15-02-1993
			AT	124601 T	15-07-1995
			DE	59202795 D1	10-08-1995
			EP	0528766 A2	24-02-1993
US 2001055642	A1	27-12-2001	US	6291009 B1	18-09-2001
			AU	7083900 A	26-11-2001
			WO	0187086 A1	22-11-2001
EP 1129626	A	05-09-2001	US	6355295 B1	12-03-2002
			AU	2477701 A	30-08-2001
			AU	2477801 A	30-08-2001
			AU	755396 B2	12-12-2002
			AU	6967100 A	30-08-2001
			BR	0004240 A	02-10-2001
			BR	0102199 A	02-01-2002
			BR	0102288 A	18-12-2001
			CA	2310296 A1	29-08-2001
			CN	1310957 A	05-09-2001
			CN	1318315 A	24-10-2001

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int—nal Application No
PCT/IB 03/00669

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 1129626 A		CN 1323547 A	28-11-2001
		DE 1129626 T1	21-02-2002
		DE 1135993 T1	18-04-2002
		DE 1129629 T1	21-02-2002
		EP 1129626 A2	05-09-2001
		EP 1135993 A2	26-09-2001
		EP 1129629 A2	05-09-2001
		ES 2165827 T1	01-04-2002
		ES 2165340 T1	16-03-2002
		ES 2165341 T1	16-03-2002
		GR 2001300063 T1	30-11-2001
		GR 2001300064 T1	30-11-2001
		HU 0005045 A2	28-02-2002
		HU 0100922 A2	28-12-2001
		HU 0100923 A2	28-12-2001
		JP 2001346544 A	18-12-2001
		NZ 509563 A	26-11-2002
		NZ 510199 A	25-10-2002
		NZ 510203 A	25-10-2002
		NZ 520357 A	28-02-2003
		NZ 520358 A	28-02-2003
		NZ 520359 A	28-02-2003
		NZ 520360 A	28-02-2003
		PL 345876 A1	10-09-2001
		PL 346197 A1	10-09-2001
		PL 346201 A1	10-09-2001
		TR 200103071 T3	21-01-2002
WO 0019839 A	13-04-2000	US 6171640 B1	09-01-2001
		AU 6503299 A	26-04-2000
		BR 9914392 A	31-07-2001
		CA 2346203 A1	13-04-2000
		CN 1326321 T	12-12-2001
		EP 1119262 A2	01-08-2001
		WO 0019839 A2	13-04-2000
		US 2001024677 A1	27-09-2001
EP 1174516 A	23-01-2002	AU 758318 B2	20-03-2003
		AU 3456800 A	16-10-2000
		EP 1174516 A1	23-01-2002
		CN 1346409 T	24-04-2002
		WO 0058492 A1	05-10-2000